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Potential risk of organic micropollutants on marine phytoplankton in the greater North Sea: integration of modelling and experimental approaches

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) of Applied Biological Sciences

Proefschrift voorgedragen tot het bekomen van de graad van Doctor in de Toegepaste Biologische Wetenschappen

Dutch translation of title:
Potentieel risico van organische micropolluenten op marine fytoplankton in de Noordzee: integratie van modellering en experimenteel onderzoek

Reference:
Everaert, G., 2015. Potential risk of organic micropollutants on marine phytoplankton in the greater North Sea: integration of modelling and experimental approaches. Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) of Applied Biological Sciences, Ghent University, Ghent, Belgium.

ISBN 978-90-5989-793-9

Le talent, ça n'existe pas. Le talent, c'est d'avoir envie de faire quelque chose.

by Jacques Brel

Voorwoord

Ook al besefte ik het op dat moment nog niet, op 8 april 2009 zou iets gebeuren met verregaande gevolgen voor de keuze van het onderwerp van mijn doctoraal proefschrift. Mijn donkergroene bolide werd ingeschreven bij de Federale Overheidsdienst Mobiliteit en Vervoer met als kenteken 140-ALG. Vanaf dat moment, dat gerust als iconisch en profetisch mag worden omschreven, maakten algen deel uit van mijn, tot dan toe studentikoze, bestaan. Prof. Peter Goethals en prof. Colin Janssen moeten dat geweten hebben. Na een sollicitatie in korte broek (het was een warme dag – verstaat u) werd deze "*speelvogel*", daarmee prof. Janssen citerend tijdens de sollicitatie, tewerkgesteld als assisterend academisch personeel.

Het moet gezegd, ik heb enkele jaren verwoede pogingen ondernomen om, tegen mijn profetie in, macro-invertebraten te bestuderen. Niet zonder resultaat, integendeel. Eigenlijk zou ik na al die jaren, met enige zin voor overdrijving, gerust een parallel boekje kunnen samenstellen over macro-invertebraten en hun relatie met de waterkwaliteit. Deze kennis werd me vakkundig ingelepeld door Koen Lock en Pieter Boets die me, tijdens mijn masterthesis, de knepen van een wetenschappelijke onderzoek leerden. Diezelfde Pieter Boets zou later een directe collega worden en samen met Ine Pauwels vanaf september 2009 deel uitmaken van ons bureautje voor de komende vier jaar. Schone tijden, hard gewerkt, veel gedeeld, lief en leed, leuke momenten met als één van de hoogtepunten (deze zijn talrijk) onze gezamenlijke deelname aan de Dodentocht in 2010. Niets voorbereid, 100km, 24 uur stappen. Het was een leuke ervaring, veel afgezien, maar dolgelukkig aan de finish. Pieter en Ine, een oprechte dank-jullie-wel voor de tijd die we samen hebben doorgebracht en nog zullen doorbrengen. Dat laatste is eerder bij een natje en droogje dezer dagen.

Mensen veranderen, soms ook van ideeën, en de macro-invertebraten achter me latend kwamen de algen opnieuw om de hoek piepen. De algen hadden het deze keer trouwens anders aangepakt, niet onsuccesvol overigens. Vermomd in de gedaante van Frederik De Laender, tot dan toe verscholen in een hoek van onze bibliotheek, kwamen ze op me af. Voor mensen die Frederik niet zo goed kennen, hij is een wetenschapper *pur sang*, begeesterd door het "*Waarom?"*. Fantastische persoonlijkheid ook, niet zonder reden ga ik niet dieper in op de nachtelijke escapades tijdens de meerdaagse teambuildings van weleer. Het is onder Frederiks vleugels dat

het algen-gerelateerd onderzoek vooruitgang boekte, los liep en toch op de rails bleef met duidelijke doelen voorop. Out-of-the-box denken, onmogelijke vragen stellen, ontmantelen, gedesoriënteerd raken, de puzzelstukken samenbrengen en interpreteren dat heb ik onthouden. Frederik, bedankt om de wetenschapsmicrobe over te dragen.

Naast Frederik liggen twee andere mensen aan de basis van dit proefschrift.
Als je niet veel tijd hebt en prof. Colin Janssen zo kort en volledig mogelijk wil omschrijven, zeg dan: passie voor de zee! Dat klopt, maar ik verkies de langere versie: luisterend oor, raadgever, klankbord en stimulator, met de daarbij horende adjectieven eerlijk en oprecht. Meer dan eens eindigde een babbel met: *"Geef er een lap op"*. Elk overleg leverde nieuwe inzichten en onderzoeksvragen, een hele menukaart als het moest, en ik mocht kiezen tussen de gerechten die je serveerde. Ik heb gesmuld van je buffet. Zeekapitein, bedankt voor de inspiratie.
Toegegeven, ik twijfelde toen prof. Peter Goethals, op het einde van academiejaar 2008-2009, polste of ik assistent wou worden. Lesgeven was een grote interesse, maar een doctoraat afwerken? De schrik om overgekwalificeerd te worden - weet u. Peter, ik ben blij dat ik op je uitnodiging ben ingegaan. Bedankt om me onder te dompelen in de wetenschappelijke wereld. Ik apprecieer de vrijheid de je me hebt gelaten, het wederzijds vertrouwen en de kansen die je hebt geboden om op (inter)nationale congressen te spreken.

Collega's van de Plateaustraat, elke discussie met jullie werkt inspirerend, ik steek daar steeds opnieuw nieuwe dingen van op. Samenwerking loont, daar ben ik van overtuigd. Ieder zijn specialiteit, maar samen één geheel. Ik hoop dat de constructieve wind blijft waaien en dat individualiteit - ten koste van het labo - wordt tegengegaan. Er is een beloftevolle generatie op komst. Werk samen, overleg en experimenteer tijdens en na de werkuren, tussen pot en pint. Pieter en Michiel V. jullie waren mijn assistent-leermeesters. Wout en Olivier, bedankt om afgelopen maanden een deel van mijn takenpakket over te nemen. Veerle en Sylvie, af en toe een chocolaatje komen halen lucht echt wel op. Marianne, merci om al mijn verzuchtingen (professioneel wel te verstaan ☺) ter harte te nemen. Aan de techniekers beneden, althans tot de verhuis (wanneer gaat die eigenlijk door?), bedankt voor jullie ondersteuning. Nancy, mijn excuses voor al die moeilijke vragen. Beste collega's, woensdag afspraak op het terras van de Vooruit?

Ik denk graag terug aan alle personen die me de afgelopen jaren steun hebben geboden, ook al begrepen ze niet altijd waar ik mee bezig was en op welke problemen ik stootte. Ik was een algenteller, niet meer of minder. Geloof me vrij, die onwetenheid was van geen tel, het allerbelangrijkste was om er gewoon te zijn, te luisteren en te steunen. Toneelvrienden, quizmakkers en voetbalkameraden, mochten we alle leeggedronken pintjes en trappisten achter elkaar zetten, we zouden een heel eind komen. Weinigen is het gegeven om zo lang samen te zijn. We hebben er al een lange weg opzitten. Dat zegt genoeg, we kennen elkaar door en door. Ik geniet ervan om bij jullie tot rust te komen.

Achter een sterke persoon huist een sterke achterban, niet in het minst vanuit familiale hoek. Meter en peter, meter Diane, peter Amand, Louis, Orpha, Luc, tantes en nonkels, neven en nichten en bij uitbreiding de hele familie die oprecht geïnteresseerd was hoe het me verging in het verre Gent. Jullie zien het, het gaat hier niet zo slecht. Bedankt voor de vele spontane vragen, de etentjes, de bezoekjes en andere kleine dingen waaruit jullie interesse bleek. Bedankt om me al die jaren thuis te laten komen.

Mathias, Eline, Wouter, papa en Lucie, wow! Recht uit het hart een oprechte dank-jullie-wel om me te laten zijn wie ik ben en om me mijn eigen weg te laten uitstippelen. Niemand kan zich inbeelden hoeveel vertrouwen jullie mij hebben geschonken, niet altijd uitgesproken, maar steeds werd ik gestimuleerd, zonder omwegen. Jullie dekken de lading van wat men zo vaak omschrijft als *"steun in goede en kwade dagen"*. Geen lege doos, maar een warm nest. Kraak een goede fles, drink die uit op mijn gezondheid. Wees trots, dat verdienen jullie. Echt.

Sigrid, woorden schieten te kort om uit te leggen wie jij bent voor mij. Heb jij de afgelopen maanden het aantal berichtjes geteld met een boodschap in de zin van: "*Zal wat later zijn. Wil nog iets afwerken. Tot straks, kus*"? Ik niet, maar ze waren ontelbaar. Telkens weer toonde je begrip. Je steun, liefde en affectie zijn van onschatbare waarde geweest om die kunstwerkje tot een goed einde te brengen. Laat ons samen van deze beeldschone overwinning genieten.

Ik wil hier bovendien nog aan toevoegen dat ik hoop dat mijn toekomstig autokenteken iets in de aard zal zijn van 1-ZPL-653. Desnoods in gepersonaliseerde vorm indien de financiën het toelaten.

Gert

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List of abbreviations

A

AIC	Akaike information criterion
AM	additive model

B

BCZ	Belgian coastal zone
BMDC	Belgian marine data center

C

CB	chlorinated biphenyl
CBB	critical body burden
CEMP	coordinated environmental monitoring programme
CHFLa	chlorophyll a

D

DAY	sampling day
DDT	dichlorodiphenyltrichloroethane
DIN	dissolved inorganic nitrogen
DSQ	dual stage quadrupole
dw	dry weight

E

EAC	environmental assessment criteria
EMODnet	European Marine Observation and Data Network
ENSO	El Nino-Southern oscillation
EPI	estimation progamme interface
EQS	environmental quality standard

G

GA(M)M	generalized additive (mixed) model
GC	gas chromatography
GLM	generalized linear model

I

ICES	International council for the exploration of the sea
ISO	International organization for standardization

K

K_{oc}	organic carbon – water partition coefficient
K_{ow}	octanol –water partition coefficient

L

LIG	light intensity
lw	lipid weight

M

MD	model deviation
MEC	measured environmental concentration
MS	mass spectrometry
MSFD	marine strategy framework directive

N

NASA-GSFC OBPG	National aeronautics and space administration – Goddard space flight center, ocean biology processing group
NAO	North Atlantic oscillation
NPZ	nutrient-phytoplankton-zooplankton
NUT	nutrient regime

O

OC	organic carbon
ORC	exposure to organic chemicals
OSPAR	Oslo and Paris conventions

P

PAH	polycyclic aromatic hydrocarbon
PAR	photosynthetically active radiation
PCB	polychlorinated biphenyl
PDMS	polydimethylsiloxane
PBT	persistent bioaccumulative and toxic
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
POP	persistent organic pollutant
PO_4	phosphate

Q

QQ	quantile - quantile

R

R	correlation coefficient
RQ	risk quotient

S

SSE	sum of squared errors

T

TBT	tributyltin
TEM	incubation temperature
TOX	body burden of POPs

U

UK	United Kingdom
UN	United Nations
UNEP	United Nations environment programme

V

VLIZ	Flanders Marine Institute
VWR	Van Waters and Rogers

W

ww	wet weight

1

General introduction and conceptual framework

1.1 Marine primary production

Phytoplankton is the basis of the pelagic food chain (Siegel and Franz, 2010) and contributes for 50% to the global primary productivity (Falkowski et al., 1998). In total, marine phytoplankton produces about 48.5 Gt carbon each year (Field et al., 1998). Marine primary production is a fundamental ecosystem process driven by solar energy and carbon dioxide availability, but also nutrient supply (e.g. nitrogen, phosphorous and microelements) is needed to sustain the process. Coastal environments are most productive (up to 450 gC m^{-2} each year) due to nutrient inputs in coastal upwelling zones and riverine and terrestrial run-off (Fig. 1.1). In temperate marine ecosystems, phytoplankton productivity is seasonally variable and closely coupled to environmental changes (Fig. 1.2). In early spring when the input of solar energy increases, nutrients are available and zooplankton grazing is low, phytoplankton blooms. After a progressive decrease of nutrient concentrations and increased zooplankton grazing in summer, phytoplankton production declines. A second smaller phytoplankton bloom may occur after replenishment of nutrient levels in late summer and autumn when the thermocline (limiting vertical mixing) breaks down (Fig. 1.2). Overall, marine primary production is driven (and limited) by the availability of light and nutrients and in a balanced ecosystem marine phytoplankton provide food for a wide range of organisms such as whales, shrimp, snails and jellyfish. Therefore, nutrient enrichment will increase phytoplankton primary production, but when too many nutrients are available eutrophication can occur. Eutrophication can result in a series of undesirable effects including changes in the structure and functioning of marine ecosystems and reduction of biodiversity. Another common effect of eutrophication is oxygen depletion, leading to fish kills and changes in fish communities, which may alter the ecosystem structure and may deplete fish stocks. Surface chlorophyll a concentrations are often used as a proxy for phytoplankton biomass and primary productivity (Fig. 1.1).

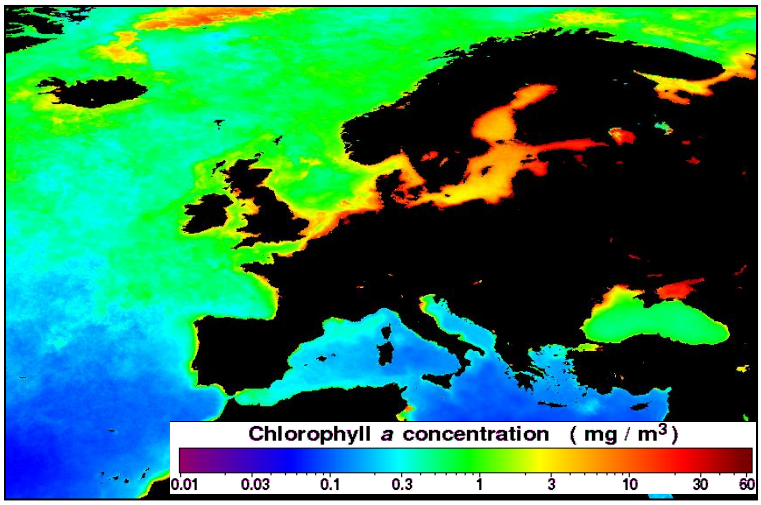


Figure 1.1 *Average annual chlorophyll a concentrations in marine waters surrounding Europe in 2007. Deep blue areas have low phytoplankton productivity, orange and red areas have high productivity. Coastal environments are most productive (Redrafted from NASA-GSFC OBPG).*

1.2 Human-induced pressure on the marine environment

There is increasing awareness of the vulnerability of the marine environment to human-induced impacts (Zacharias and Gregr, 2005). With a global population growth of 0.77% per year (UN, 2004), the anthropogenic pressure on the marine environment has drastically increased during the last decades (Dachs and Mejanelle, 2010). Oil and gas extraction, coastal engineering works, fishing and chemical pollution are considered key drivers of marine ecosystem change (Zalasiewicz et al., 2008; Halpern et al., 2008; Crain et al., 2009). Globally about 41% of the oceans and seas are impacted to a high or very high degree (Halpern et al., 2008). Projections of future change indicate that marine ecosystems will continue to be affected by anthropogenic-driven changes of which the rate will increase (Howard et al., 2013). In this context, past decades focus in eco(toxico)logical research has been mainly on the effects of ocean warming and ocean acidification on marine ecosystems (e.g. Portner, 2008; Harvey et al., 2013). As such, to date the combined impact of ocean warming and acidification and ecotoxicological effects due to chemicals pollution are poorly understood (Holmstrup et al., 2010). This is problematic because currently about 250,000 man-made chemicals are present in the marine environment (OSPAR, 2010) and future projections estimate an increased production of chemicals of 3% per year in the coming decades (Wilson and Schwarzman, 2009). Understanding the impact of these changing environmental conditions on the marine primary production is crucial as alterations in the phytoplankton community can affect entire marine ecosystems (Burkiewicz et al., 2005).

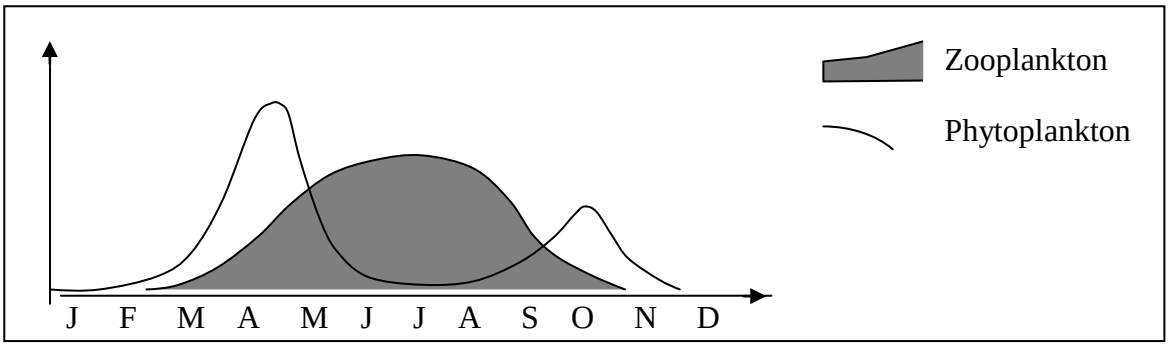


Figure 1.2 *Seasonal variability of phyto- and zooplankton production in temperate marine ecosystems (Cushing, 1975).*

1.3 Chemical pollution

1.3.1 Sources, transport and partitioning of chemical pollution

Chemicals, such as organic contaminants, enter the marine environment through various routes from point and non-point sources (Fig. 1.3). Most organic contaminants are synthetic chemicals either intentionally used in industrial processes and agricultural applications or unintentionally released from traffic and waste. Examples include emissions from (incomplete) combustion of fossil fuels used for heating and for transport or unintentionally produced by-products of synthetic organic substances (e.g. plastics and pesticides; Van Leeuwen and Vermeire, 2007). Some organic chemicals are of natural origin, e.g. from volcanic eruptions or wildfires (Fig. 1.3).

Both natural and anthropogenic organic chemicals are released into air, water and land, globally distributed via air and ocean currents and eventually deposited in the aqueous, soil and biotic compartment even in remote environments far away from direct sources (Fig. 1.3). The behavior and fate of chemicals in the environment is determined by their chemical and physical properties and by the nature of the environment (see further), but at global scale the marine environment is the main receptor of organic contaminants (Law et al. 2010).
The distribution, deposition and remobilization of organic chemicals in the marine environment does not only depend on air and ocean currents, but also on the physical-chemical properties of the organic chemicals. Two well-known examples in this context are the grasshopping effect and the latitudinal fractionation of organic chemicals. The grasshopping effect is a process by which organic chemicals are transported from warmer to colder regions of the Earth (e.g. poles and mountains). The grasshopping effect is the main reason for the relative high concentrations of persistent organic pollutants (POPs) in cold ecosystems (Vallack et al., 1998). The latitudinal fractionation process is related to the grasshopping effect and is the compositional shifts of organic chemicals along latitudinal gradients. When organic chemicals of both high and low volatility are transported to remote cold regions such as the Arctic, the more volatile chemicals become relatively more abundant with increasing latitude. The main reason for this is that the partitioning between air and water shifts as temperature drops (Bustnes et al., 2012). Overall, the distribution of organic chemicals does not only depend on the ocean and air current but also on the type chemicals involved.

The marine environment consists of four main compartments (i.e. air, water, sediment and biota) and organic chemicals tend to migrate between these compartments in case they are not in equilibrium. Some organic chemicals are ionizable compounds and have been recognized as emerging pollutants in the marine environment. Typical examples of such compounds are pharmaceuticals and personal care products with amide, acids and amines as functional groups. Most important factors influencing the partitioning of these ionizable organic chemicals are intermolecular ion-ion interactions (i.e. hydrogen bonding, van der Waals forces, cation exchange, cation bridging and surface complexes). By contrast, the partitioning of organic contaminants, such as polychlorinated biphenyls (PCBs), between environmental compartments mainly depends on the physical-chemical properties of the chemicals involved. Organic chemicals with a high vapor pressure and low water solubility will mainly be found in the air when an equilibrium situation is established. Apolar substances with high K_{ow} (octanol-water partitioning coefficient) and high K_{oc} (organic carbon - water partitioning coefficient) will tend to accumulate in biological tissues and sediments (Jones and de Voogt, 1999) and can enter the food web by accumulating from the freely dissolved phase into phyto- and zooplankton. When concentrations of organic chemicals in an organism exceed concentrations in the consumed prey, biomagnification occurs (Van Leeuwen and Vermeire, 2007). Due to biomagnification, organic chemicals can achieve high concentrations in biological tissues even though chemical exposure in the water compartment may appear limited. Hence if biomagnification occurs, highest

concentrations of persistent organic pollutants (POPs) are typically found in top-predators such as fish-eating birds and marine mammals and can be several orders of magnitude higher than the freely dissolved POP concentrations (Fig. 1.3; Table 1.2). Typical examples of such chemicals include many of the first generation organochlorine insecticides such as dichlorodiphenyltrichloroethane (DDT) and industrial chemical products or byproducts including PCBs. DDT was one of the most widely used insecticides in the 1950s. Small amounts of DDT in micro-organisms magnified in the biological tissues of predatory birds and induced eggshell thinning. As the eggshell broke due to the mother's weight, predatory birds were not able to reproduce and their population sharply decreased during the time of heavy DDT use (Holm et al., 2009). Overall, organic chemicals of most ecological concern are those with persistent, toxic and bioaccumulative (PBT) properties. Notwithstanding low freely dissolved concentrations, these chemicals can achieve concentrations high enough to exert ecotoxicological effects (see further). Polychlorinated biphenyls and chlorinated pesticides (Box 1.1) serve as model compounds for POPs present in the marine environment and have a relatively long (> three decades) monitoring history.

1.3.2 Monitoring of chemical pollution

Monitoring is needed to detect, quantify and assess the trends of chemical concentrations in the marine environment. Indeed, monitoring is the first step to detect pollution-induced ecosystem changes. Monitoring activities over several decades have revealed the ubiquitous presence of organic chemicals in the marine environment (Table 1.2). A non-exhaustive list of PCB concentrations measured in the marine environment was compiled from scientific literature (Table 1.2). Based on this list, three general conclusions about the presence of organic chemicals the marine environment can be drawn. First, PCBs are widespread and detected in all environmental compartments. Second, although monitoring activities occur mainly in coastal waters and inside harbours, concentrations of PCBs in the aqueous phase and in the sediment compartment of harbours are higher than those in open sea. For example, Gioia et al. (2008) reported that concentrations of the sum of seven PCBs in the open Atlantic Ocean are between 0.071 $pg.L^{-1}$and 1.70 $pg.L^{-1}$. Concentrations of PCBs in estuaries and harbours are up to three orders of magnitude higher than in open sea (e.g. Emelogu et al., 2013; Schaanning et al., 2011). Third, PCB concentrations in biota increase with increasing trophic level, demonstrating biomagnification. Examples include the concentrations of PCB in zooplankton in Newfoundland (Canada) of 0.7 $\mu g.kg\ ww^{-1}$ (Ray et al., 1999) and PCB concentrations in dolphins along the Canary islands (Spain) between 300 and 33,000 $\mu g.kg\ ww^{-1}$ (Carballo et al., 2008; Table 1.2). In line with international legislation (Box 1.2), also along the Belgian coast and in the Western Scheldt estuary considerable monitoring efforts have been performed to quantify the concentrations of POPs. In Figure 1.5 recently monitored PCB concentrations in four Belgian harbours (Antwerp, Nieuwpoort, Ostend and Zeebrugge) and at the Belgian Coastal Zone (BCZ) are summarized.

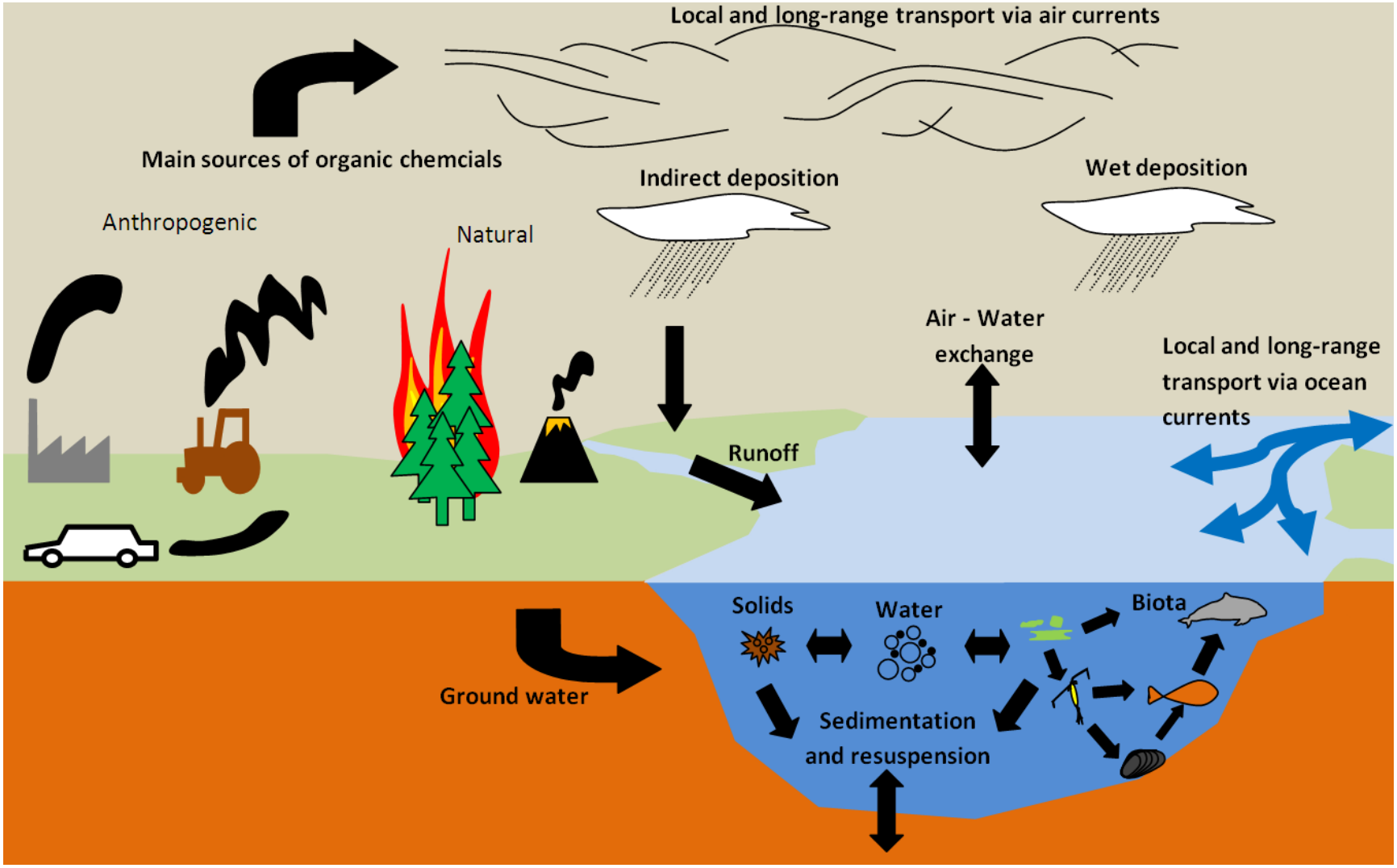


Figure 1.3 *Atmospheric emissions, transport and deposition/remobilization processes of persistent organic pollutants (Based on EPA, 2000).*

Box 1.1 Polychlorinated biphenyls and chlorinated pesticides

Polychlorinated biphenyls (PCBs) are synthetic chemicals used as insulators and cooling fluids in transformers, in the oil of electric capacitors, as hydraulic fluids and plasticizers in paints. PCBs have been released into the environment through open burning and incomplete incineration, by vaporization (from paints, coatings and plastics), by leakage into surface waters, by dumping in landfills and oceans. It is estimated that globally between 1.2 and 1.3 million metric tonnes of PCB compounds have been produced (Giesy and Kannan, 1998). Due to concerns about the environmental impact of PCBs, production was stopped in the late 1970s in Western Europe and North America and in the early 1990s in Eastern Europe and Russia (Fig. 1.4).

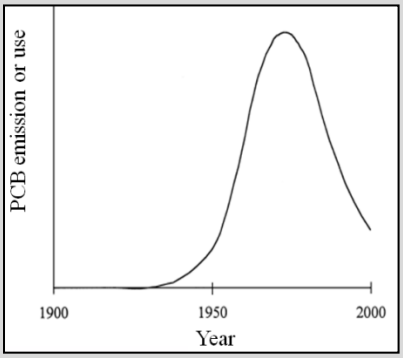


Figure 1.4 *Relative emissions to the environment of polychlorinated biphenyls (PCBs) between 1990 and 2000 (Redrafted from Jones and de Voogt, 1999.)*

PCBs are stable chemicals, with low volatility at normal temperature. They are soluble in most organic solvents but are almost insoluble in water. They are environmentally hazardous due to their extreme resistance against chemical and biological breakdown by natural processes in the environment. Significant amounts of PCBs are stored in the environment and with decreasing primary emissions it is expected that (re)cycling of secondary sources will become more important for potential environmental risks. PCBs produce a variety of ecotoxicological effects ranging from the disruption of photosynthesis in phytoplankton to impaired reproduction in higher animals. In chapter VI focus is, apart from PCBs, also on four chlorinated pesticides (i.e. p,p'-dichlorodiphenyltrichloroethane, alpha-hexachlorocyclohexane, gamma-hexachloro-cyclohexane and hexachlorobenzene). An overview of the most important physical-chemical properties is given in Table 1.1.

Table 1.1 *Polychlorinated biphenyls (PCBs) and pesticides considered in this study. Log Kow is the octanol-water partitioning coefficient. BCF is the bioconcentration factor. Data were obtained from EPI Suite™ v4.10 application (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm).*

Chemical compound	Code	Log K_{ow} [(a)]	BCF [(b)]	Log K_{oc} [(c)]	Biotransformation rate [(d)]	Molecular weight [(e)]
2,4,4'-Trichlorobiphenyl	CB28	5.69	9143	4.178	0.0025	257.5
2,2',5,5'-Tetrachlorobiphenyl	CB52	6.34	18670	4.438	0.00125	292.0
2,2',4,5,5'-Pentachlorobiphenyl	CB101	6.98	54910	4.831	0.00085	326.4
2,3',4,4',5-Pentachlorobiphenyl	CB118	6.98	44790	5.008	0.00075	326.4
2,2',3,4,4',5'-Hexachlorobiphenyl	CB138	7.62	31220	5.185	0.000625	360.9
2,2',4,4',5,5'-Hexachlorobiphenyl	CB153	7.62	22000	5.356	0.0005	360.9
2,2',3,4,4',5,5'-Heptachlorobiphenyl	CB180	8.27	12260	5.644	0.00035	395.3
p,p'-dichlorodiphenyltrichloroethane	DDTPP	6.79	16840	5.893	0.001	354.5
alpha-hexachlorocyclohexane	HCHA	4.26	250.4	3.593	0.01	290.8
gamma-hexachlorocyclohexane	HCHG	4.26	250.4	3.593	0.01	290.8
hexachlorobenzene	HCB	5.86	2803	4.239	0.009	284.8

[(a)]L water L octanol^{-1}; [(b)]L kg wet weight^{-1}; [(c)]L water kg sediment^{-1}; [(d)]d^{-1}; [(e)]g mol^{-1}

Monitoring data were obtained in the scientific literature and covered three environmental compartments (water, sediment and biota) and three trophic levels. In addition, we provide, for each environmental compartment, environmental quality criteria (see further). Overall, global patterns described above are also valid for the BCZ and the Western Scheldt estuary (Fig. 1.5).

Routine monitoring activities particularly focus on -so called- priority chemicals (e.g. PCBs; OSPAR, 2010) which are chemicals that have been prioritized in legislative frameworks (Box 1.2). As such, in comparison to the multitude of chemicals present in the marine environment (Dachs and Mejanelle, 2010), only few (groups of) chemicals are routinely monitored. By doing so, several potentially harmful chemicals may be overlooked and the real chemical cocktail present in marine waters is unknown. The fate of emerging chemical contaminants (Hutchinson et al., 2013) such as pharmaceuticals (Claessens et al., 2013) and personal care products (Richardson et al., 2005) is poorly understood. Overall, the chemical composition - in terms of chemical pollution - of marine waters is poorly studied as monitoring mainly focuses on the detection and measurement of priority substances. For a limited number of substances, such as PCBs (Table 1.2; Fig. 1.5), polycyclic hydrocarbons (PAHs) and pesticides relatively large sets of monitoring data are available.

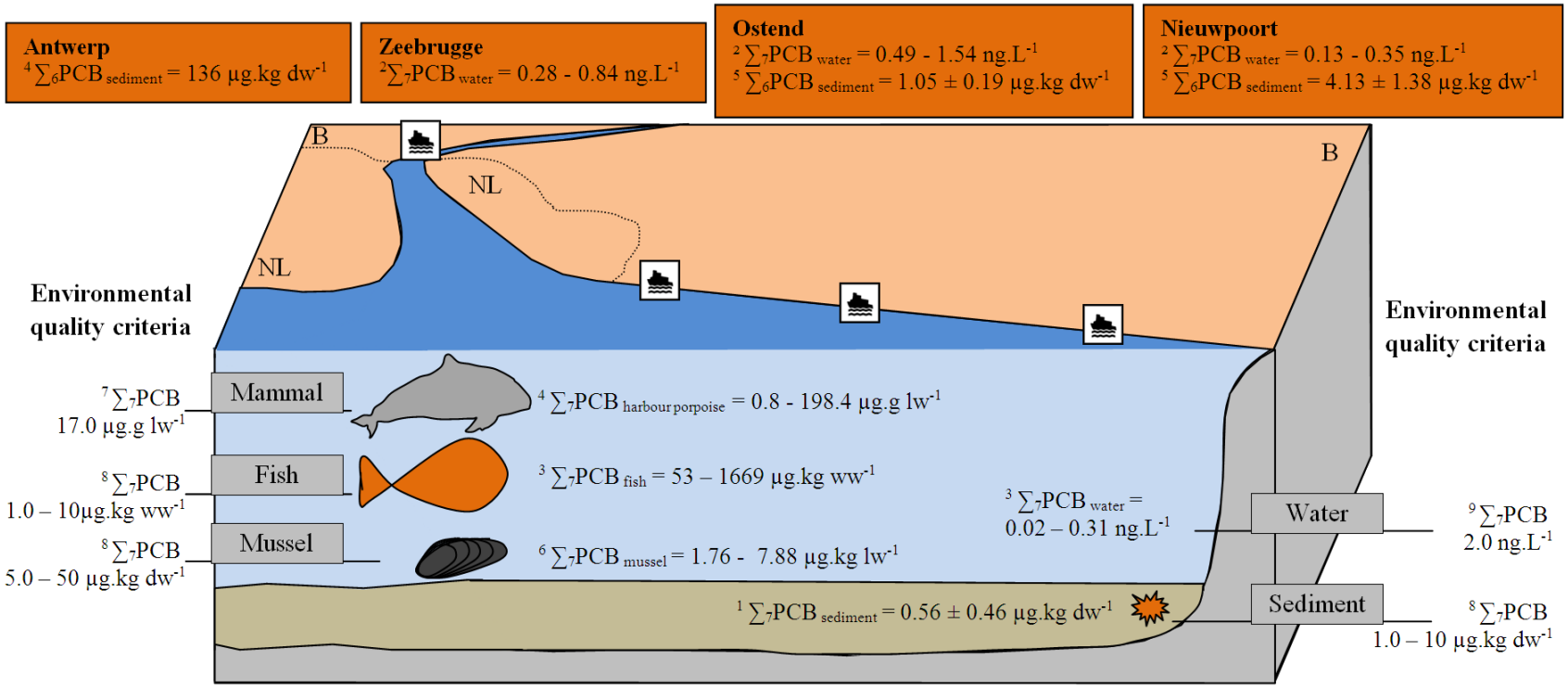


Figure 1.5 *Concentrations of polychlorinated biphenyls (PCBs) in four Belgian harbours and on the Belgian Coastal Zone. The reported ranges represent the sum of concentrations of six ($\sum_6$) or seven ($\sum_7$) PCB congeners. Depending on the environmental compartment, concentrations are reported as dry weight (dw), wet weight (ww) or lipid weight (lw). Concentrations based on: (1) Everaert et al., 2014 (sampling campaign in 2010); (2) Monteyne et al., 2013 (sampling campaign in 2007-2009); (3) Voorspoels et al., 2004 (sampling campaign in 2001); (4) Van Ael et al., 2012 (sampling campaign in 2010); (5) Danis et al., 2004 (sampling campaign in 2001); (6) Danis et al., 2006 (sampling campaign in 2001); (7) Kannan et al., 2000; (8) Environmental assessment criteria (EAC); (9) Environmental quality standard (EQS).*

Table 1.2 *Non-exhaustive overview of concentrations of polychlorinated biphenyls in three marine environmental compartments. (1) Gioia et al. (2008); (2) Iwata et al. (1993); (3) Emelogu et al. (2013); (4) Yan et al. (2008); (5) Guan et al. (2009); (6) Schaanning et al. (2011); (7) Roach et al. (2009); (8) Monteyne et al. (2013); (9) Garcia-Flor et al. (2005); (10) Webster et al., 2011; (11) Russell et al., 2011; (12) Biselli et al., 2005; (13) Gomez-Lavin et al., 2011; (14) Nunes et al., 2011; (15) Stronkhorst & van Hattum et al., 2003; (16) Danis et al., 2004; (17) Ray et al., 1999; (18) Van Ael et al., 2012; (19) Carlsson et al., 2011; (20) Bodin et al., 2014; (21) Vorkamp et al., 2008; (22) Carballo et al., 2008; (23) McKinney et al., 2011.*

Matrix and location	Number of samples	Group of congeners	Concentration	Reference
PCB - Water				
Open water				
Atlantic Ocean	15	$\sum_7$PCBs	0.071 – 1.70 [a]	(1)
South China Sea	68	$\sum_{40}$PCBs	0.01–0.033 [b]	(2)
Estuary				
Scotland, Forth estuary	5	$\sum_7$PCBs	0.07 – 0.13 [b]	(3)
USA, Hudson estuary	23	$\sum_{90}$PCBs	0.37 – 1.60 [b]	(4)
China, Pearl river delta	16	$\sum_{20}$PCBs	0.12 – 1.47 [b]	(5)
Norway, inner Oslofjord	24	$\sum_7$PCBs	<0.01 - 0.20 [b]	(6)
Harbor				
Australia, Sydney harbor	4	$\sum_{12}$PCBs	0.02 - 0.54 [b]	(7)
Belgium, coastal harbors	26	$\sum_{14}$PCBs	0.03 – 3.10 [b]	(8)
Spain, Barcelona harbor	6	$\sum_{41}$PCBs	0.30 – 7.62 [b]	(9)
Spain, Banyuls harbor	4	$\sum_{41}$PCBs	7.14 – 15.8 [b]	(9)
PCB - Sediment				
Coastal waters				
Seas around Scotland	466	$\sum_7$PCBs	0.17 – 0.81 [c]	(10)
Scotland	43	$\sum_7$PCBs	< 0.02 – 3.45 [c]	(11)
Baltic Sea	7	$\sum_{10}$PCBs	1.87 – 105 [c]	(12)
Estuary				
Spain, North Atlantic coast	18	$\sum_6$PCBs	0.39 – 4.06 [c]	(13)
Portugal, multiple estuaries	31	$\sum_{12}$PCBs	0.03 – 8.68 [c]	(14)
Scotland, Clyde estuary	206	$\sum_7$PCBs	0.83 – 26.0 [c]	(10)
Harbor				
The Netherlands, harbours	257	$\sum_7$PCBs	< 1.00 – 456 [c]	(15)
Belgium, Nieuwpoort	3	$\sum_6$PCBs	4.13 ± 1.38 [c]	(16)
Belgium, Ostend	3	$\sum_6$PCBs	1.05 ± 0.19 [c]	(16)
PCB - Biota				
Canada, Newfoundland	25	$\sum_{22}$PCBs $_{zooplankton}$	0.7 [d]	(17)
Belgium, Scheldt estuary	6	$\sum_6$PCBs $_{mussel}$	119 – 759 [d]	(18)
Sweden	9	$\sum_{13}$PCBs $_{herring, sprat}$	7.6 – 20.0 [e]	(19)
Scotland, Clyde estuary	291	$\sum_7$PCBs $_{plaice}$	543.0 – 1950 [e]	(10)
Seas around Scotland	116	$\sum_7$PCBs $_{plaice}$	183 – 354 [e]	(10)
France, Gironde estuary	8	$\sum_7$PCBs $_{sole}$	982.0 - 2852 [e]	(20)
Denmark, Greenland	96	$\sum_{10}$PCBs $_{ringed\ seals}$	650 [e]	(21)
Spain, Canary islands	11	$\sum_{11}$PCBs $_{dolphins}$	300 – 33,000 [d]	(22)
Arctic environment	165	$\sum_{74}$PCBs $_{polar\ bears}$	1,800 – 10,500 [e]	(23)

[a] $pg.L^{-1}$ (based on 700L concentrated seawater); [b] $ng.L^{-1}$; [c] $\mu g.kg\ dw^{-1}$; [d] $\mu g.kg\ ww^{-1}$; [e] $\mu g.kg\ lw^{-1}$

Box 1.2 From programmes and conventions to legislation: a brief historical background
In the 1970s, as it became clear that POPs remain in the environment for long periods, are globally distributed, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife, multiple large-scale international and regional conventions and management programmes were initiated. The overall aim of these conventions and programmes was/is to stop the production and use of POPs and to provide a framework to frequently monitor the pollution status of the marine environment. To do so, specific groups of substances were selected, i.e. the so-called priority legacy substances. These substances have hence been routinely monitored in the marine environment since the 1980s.
Criteria for assessing the environmental quality in relation to measured concentrations of chemicals have been established such as the environmental quality standards (EQS) and the environmental assessment criteria (EAC). As it is not within the scope of this paper to list all the international conventions and organization, we only discuss those frameworks that are most relevant in the context of this research.

The key objective of the **Stockholm Convention** (2001), signed by 152 governments, is to protect human health and the environment from persistent organic pollutants (POPs). In implementing this convention, governments have to take measures to eliminate or reduce the release of POPs into the environment. The Baltic Marine Environment Protection Commission or the **Helsinki Commission** aims to protect the marine environment of the Baltic Sea from all sources of pollution and to restore and protect marine ecosystems. Monitoring and assessment of emissions from both point sand diffuse sources are an integral part of the convention. The tasks of the Convention for the Protection of the Marine Environment of the North-East Atlantic or OSPAR (www.ospar.org) were originally handled by two individual commissions: the Oslo Commission (Convention for the Prevention of Marine Pollution by Dumping from Ships and Airplanes) and the Paris Commission (Convention for the Prevention of Marine Pollution from Land-Based Sources). In 1992, the **OSPAR Commission** was initiated under its current form with the general aim of the cessation of discharges, emissions and losses of hazardous substances by 2020. The key objective is to achieve concentrations in the marine environment close to background values for naturally occurring substances and close to zero for man-made synthetic substances.

The **Marine Strategy Framework Directive** (MSFD) was adopted in June, 2008 (Directive 2008/56/EC) and aims to achieve a good environmental status of European marine waters by 2020. In this context the term “good environmental status” means “the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations.” As such, chemical contaminants may not give rise to pollution effects altering the production, structure and functioning of the marine environment.

1.3.3 Spatiotemporal trends of chemical pollution

Spatial and temporal trend detection is important to determine increases or decreases of chemical pollution. Global monitoring indicates that concentrations of legacy organic contaminants such as PCBs and pesticides decreased over the past decades (Law, 2014). Following the production ban of PCBs in the 1970s (Fig. 1.4), decreasing time trends of PCB were reported in air (e.g. Hung et al. 2010, Schuster et al., 2010; Venier et al., 2012), seawater (e.g. Gioia et al., 2008) and sediment (e.g. Konat and Kowalewska, 2001, Johannessen et al., 2008 and Hong et al., 2010). Concentrations of PCBs in biota also exhibit a decreasing trend. For example, Ross et al. (2013) observed an 81% decrease of PCB concentrations in harbor seals from the Salish Sea in North America between 1984 and 2003. Similarly, Roose et al. (1998) and Wejis et al. (2012) found decreasing PCB concentrations in cod and in harbor porpoises in the BCZ. In some particular cases, PCB concentrations seem to achieve a status quo (Vorkamp et al., 2011; Law, 2014).

The main reason for the generally decreasing PCB time trends is that the primary production and use of PCBs has been restricted since the 1970s (Fig. 1.4). However, after decades of primary emissions, reservoirs of PCBs have accumulated in soil, water and biota. These reservoirs can be remobilized due to changes in temperature and soil organic matter (Cabrerizo et al., 2013) or physical disturbances due to sand or gravel extractions and dredging activities (Martins et al., 2012). Recently, Ma et al. (2011) used long time series of air concentrations and suggested that Arctic seawater, snow and soil are important secondary sources remobilizing POPs and modulating the trends of legacy pollutants. So, as primary emissions cease, secondary emissions from residues deposited in water, soil and biota may significantly influence the fate of PCBs in the environment (Nizzetto et al., 2012).
The BCZ and the Western Scheldt estuary are globally amongst the most industrialized and urbanized regions. Due to historical release from industry and run off from the hinterland past decades, sediment, water and biota have been accumulating large amounts of PCBs. Currently, high concentrations of PCBs are buried in sediments and accumulated in biota in this region (Fig. 1.5; Voorspoels et al., 2004). Recently, Breivik et al. (2007) predicted decreasing long-term emissions of PCBs for the BCZ and the Western Scheldt estuary. As such, secondary emissions from historically polluted environmental compartments may play an increasingly important role on the fate of PCBs. However, the relative impact of primary and secondary sources on the spatiotemporal trends of PCB concentrations in the BCZ and the Western Scheldt estuary has not been studied so far. As such, it is unclear whether the globally detected downward trends are buffered by secondary emissions. Therefore, the relation between primary and secondary emissions and their influence on the spatiotemporal trends of PCBs in the BCZ and the Western Scheldt estuary needs further investigation.

Elimination of PCBs under (inter)national regulations reduced primary emissions (Fig. 1.4), but secondary emissions may continue from residues deposited in soil, water and biota (Bidleman et al., 2013). One of the underlying biogeochemical processes controlling these secondary emissions is the biological pump (Nizzetto et al., 2012). The main principle of the biological pump is that POPs are taken up in marine phytoplankton (Gasol et al., 1997) and

are subsequently transferred to deeper water and sediment (Dachs et al., 1999; Jaward et al., 2004). When phytoplankton blooms, dissolved-phase POP concentrations in surface water are depleted (Skoglund et al., 1996), which in turn promotes the air-to-water flux of lipophilic POPs (Galban-Malagon et al., 2012). The role of the biological pump (Nizzetto et al., 2012) in the sequestration of lipophilic POPs was recently examined in the Atlantic and Arctic oceans (Jaward et al., 2004; Galban-Malagon et al., 2012) and in the Mediterranean Sea (Berrojalbiz et al., 2011). These studies report that seasonal changes in phytoplankton biomass result in seasonally variable POP concentrations. Although these studies provide valuable quantifications of the fluxes of POPs between environmental compartments, noteworthy drawbacks are that data are mainly collected in open sea and in one phytoplankton growth season. Open sea concentrations of PCBs are typically lower than concentrations in coastal waters and in estuaries (Table 1.2). As such, the impact of the biological pump on the fate of POPs close to industrialized and urbanized regions is identified as a knowledge gap. Secondly, the impact of the biological pump on large spatiotemporal scales is currently unknown.

1.4 Ecotoxicological effects of chemical pollution

1.4.1 Environmental risk assessment

Ecotoxicological effects of persistent organic pollutants (POPs) have often been assessed using standardized laboratory toxicity tests (Walker et al., 2001). In this deterministic approach potential ecotoxicological effects are assessed by exposing (a group of) test organisms to a concentration range of a chemical under controlled circumstances (i.e. effect assessment). The purpose is to quantify the relation between the concentration of a chemical and the effect caused by this chemical on an organism. The result is called a concentration-response relationship and is central in ecotoxicology. In a regulatory context, results of standardized laboratory toxicity tests have been used to infer a predicted no effect concentrations (PNEC; Fig. 1.6) and environmental quality criteria such as environmental assessment criteria (EAC) and environmental quality standards (EQS) for legislative purposes (Box 1.2). To do so, an assessment factor approach or, when sufficient data is available, a statistical extrapolation method such as species sensitivity distributions (SSDs) have been used (EC, 2003).

In ecological risk assessment, PNECs are compared with the expected environmental concentrations, quantified based on measurements (measured environmental concentration, MEC) or, if no monitoring data are available, model predictions based on the chemical properties of the target substance and the surrounding environmental conditions (predicted environmental concentrations, PEC; Fig. 1.6). The ratio between the PEC (based on exposure assessment) and PNEC (based on effect assessment), indicates the potential risk to the environment. If this value is larger than 1, the PEC exceeds the PNEC and the chemical poses a possible risk for the environment (Fig. 1.6).

The ratio between the PEC and PNEC, often referred to as ‘risk quotient’ (RQ; Fig. 1.6), has been often used in environmental risk assessment of organic chemicals. Recent examples of this type of ecological risk assessment in the BCZ include studies of Ghekiere et al. (2013)

and Claessens et al. (2013). Ghekiere et al. (2013) reported potential risks for tributyltin (TBT), polybrominated diphenylethers (PBDEs), PCBs and five PAHs in the water compartment and for TBT and PCBs in the sediment compartment. Claessens et al. (2013) concluded no direct risk of pharmaceuticals observed in the Belgian marine environment. Important to point out in this context is that there is no causal relationship between RQs higher than one and real ecotoxicological effects (Wu et al., 2008; Brown et al., 2013). The main reason is that many of the established criteria are associated with a large degree of uncertainty (Wu et al., 2008) and rely on a large number of assessment factors (EC, 2003). EAC for example, are usually expressed as a -one order of magnitude- range around the actual threshold. Therefore, it is useful to explore the origin of the EAC, to verify how EAC were defined and how the EAC-related uncertainty may influence the environmental risk assessment (risk vs. no risk). Moreover, although spatiotemporal trends of organic chemicals have been relatively frequent studied in different parts of the world (cfr. 1.3.3), spatiotemporal trends of risk quotients taking into account the uncertainty around the EAC are not available in scientific literature.

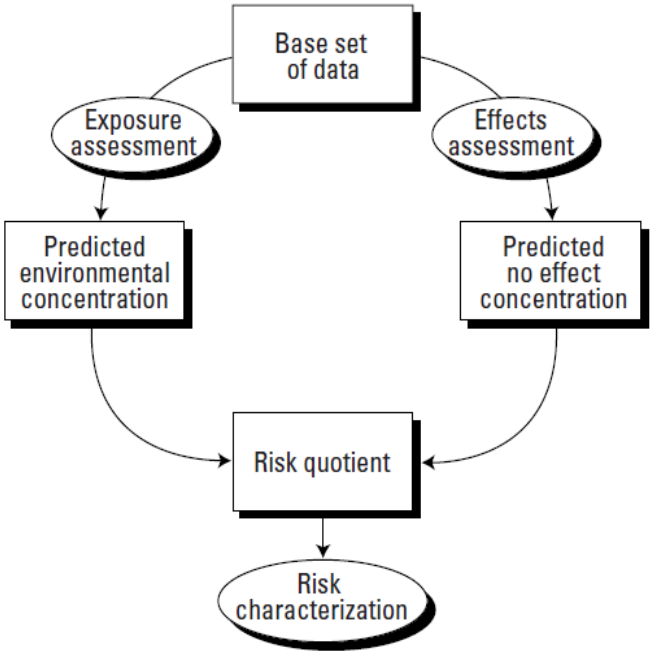


Figure 1.6 *Elements of an environmental risk assessment: hazard identification, effects assessment, exposure assessment and risk characterization (Redrafted from van Leeuwen & Vermeire, 2007).*

1.4.2 Mixture toxicity

Marine ecosystems are exposed to increasingly complex mixtures of organic chemicals (Dachs and Mejanelle, 2010), but knowledge about their ecotoxicological effects is limited to few priority substances (OSPAR, 2010). Indeed, most laboratory-based ecotoxicological research with organic chemicals focused on PCBs, PAHs and pesticides (Holmstrup et al., 2010). Therefore, the translation of laboratory-observed ecotoxicological effects towards the real marine environment is often difficult. For example, effect concentrations for PCBs (e.g. Plesha et al., 1988; Chapman et al., 1996; Leitao et al., 2003; Lee et al., 2006) are several

orders of magnitude lower than the measured environmental concentrations (Table 1.2) and might seem too low to exert an individual ecotoxicological effect. However, a complex mixture of hydrophobic chemcials is present in the marine environment and their combined effect may induce ecotoxicological effects through combined toxicity (Petersen et al., 2014). So, in addition to the frequently monitored and assessed priority substances, also other organic chemicals can cause (joint) effects. As the aim of ecotoxicology is to develop environmentally realistic causal relationships between exposure concentrations and observed effects, chemical mixture effects should be integrated (Backhaus et al., 2003). A major limitation hampering the study of mixtures effects is that the knowledge about the chemical composition of marine waters in terms of chemical pollution is poor (cfr. 1.3.2). Although there are some exceptions (e.g. Echeveste et al., 2010) most studies solve this problem by using artificial mixtures of organic chemicals. However, these mixtures are only an approximation of the real mixtures of chemicals and may lack environmental realism (Backhaus et al., 2003).

To overcome the problem that effect concentrations are often considerably higher than the ambient concentrations and to test more environmentally relevant mixtures of organic chemicals, new experimental strategies have been developed. These strategies should facilitate the translation of laboratory-observed ecotoxicological effects towards the true marine environment. In this context, passive samplers were recently applied to assess ecotoxicological effects of organic chemicals (e.g. Lohmann et al., 2012; Booij et al., 2013; Emelogu et al., 2013). The main advantage of using passive samplers is that environmentally relevant chemical mixtures can be tested and that test concentrations represent realistic environmental concentrations of organic chemicals averaged over a relatively long period (Lohmann et al., 2012). Moreover, when using loaded passive samplers as passive dosing devices, exposure concentrations will remain constant over the entire test duration (Claessens, 2013). Recently, passive samplers were used in bioassays to determine the toxic pressure of organic contaminants on primary producers along the Dutch coast (Booji et al., 2013). In the Forth estuary in Scotland, Emelogu et al. (2013) used the extract of passive samplers to quantify the ecotoxicological effect of chemical stressors in an algal growth inhibition test. Information along the Belgian coast relies on a study of Claessens (2013) in which a passive sampler-passive dosing study was performed in three harbors and in open sea. Results obtained depended on the location of deployment and the time of deployment of the passive samplers. As such, additional research is needed to test the ecotoxicological effect of realistic environment mixtures of organic chemicals present in Belgian coastal waters.

1.4.3 Natural variability and ecotoxicological effects

The ultimate goal when performing ecotoxicological research is to quantify the chemicals' effect on real ecosystem structures and functions. However, due to practical reasons, ecotoxicological research is often performed under controlled laboratory conditions. In such laboratory experiments model species are often kept in optimal conditions (temperature, nutrients, light etc.) to isolate the effects of the chemical in question (e.g. Walker et al., 2001). The main drawback of those standard test conditions is that the conversion of laboratory-based conclusions towards field conditions is hampered as organisms rarely experience those

optimal conditions in their natural settings (Holmstrup et al., 2010). Therefore, ecotoxicological studies of organic chemicals ideally integrate field data and lab-scale studies under controlled conditions. A main advantage of using field data is that the natural background variation of important bottom-up drivers of marine ecosystems (cfr. 1.2) are implicitly included in the results that are obtained. For example, seasonal variation in the physical-chemical properties of soil, water and air may influence the bioavailability of organic chemicals and causes different effects in different exposure scenarios (e.g. Newman and Unger, 2003). Likewise, natural variability in water temperature and nutritional status may influence the ecotoxicological effect of organic chemicals. A negative aspect of using field data is that sampling methodologies and analytical instruments may evolve, hence introducing extra (but unwanted variability (noise)) in the data. To take advantage of existing field data for ecotoxicological research, initiatives such as the Ecosystem Data Online Warehouse (http://ecosystemdata.ices.dk/), developed by the International Council for the Exploration of the Sea (ICES) to collect and to maintain marine biological, oceanographic and contaminants data are highly needed. Such initiatives provide reliable and comprehensive data useful for other scientific purposes. Next to ICES, also other data centers such as the Belgian Marine Data Center (BMDC), the datacenter division of Flanders Marine Institute (VLIZ) and the European Marine Observation and Data Network (EMODnet) archive marine data. In conclusion, to quantify chemicals' effect on real ecosystems, ecotoxicological research should integrate lab-scale experiments under controlled conditions and field data including the natural background variation.

By using existing field data, the relative contribution of natural drivers and organic chemicals to marine processes (and eventually ecosystems) can be quantified. A typical example in this context includes the growth dynamics of marine phytoplankton. Marine phytoplankton growth is mainly driven by light, nutrients and temperature dynamics (Boyd and Hutchins, 2012) explaining about 65% of the variability observed in the phytoplankton biomass dynamics (Irwin and Finkel, 2008). However, given the natural variation of light, temperature and nutrients, it is unclear whether and how much chemical pollution affects phytoplankton dynamics. Indeed, to date the relative contribution of organic chemicals to the phytoplankton growth dynamics has not been quantified yet. To fill this knowledge gap, long-term existing data of both chemical pollution and natural drivers can be used. By doing so, the natural driven phytoplankton changes can be separated from pollution-driven changes, which is a crucial element when using field data in an ecotoxicological context (Law et al., 2010). By quantifying the relative (compared to natural drivers) impact of organic chemicals on the growth dynamics of marine phytoplankton, a first step is taken to provide insight in the effect of chemical pollution on marine ecosystems under naturally occurring environmental changes.

1.5 Problem formulation – rationale for this thesis

Marine phytoplankton, being the basis of the pelagic food web, is an essential part of marine ecosystems. Alterations of phytoplankton productivity due to changing environmental conditions can have far-reaching consequences for marine ecosystems. A complex mixture of organic chemicals is present in marine waters, but routine monitoring focuses on the detection and measurement of priority substances only. As such, neither the chemical composition of marine waters, nor the ecotoxicological effects of organic compounds present in marine waters are known. To date the effects of changing environmental conditions, including the presence of organic chemicals, on marine primary production are poorly studied. Although we know that a complex mixture of organic chemicals is present in marine waters, the related ecotoxicological risks are largely unknown.

In this context, using model-based analyses of existing data and experimental laboratory work (Fig. 1.7), this research aimed to:

- infer spatiotemporal trends of the presence of organic chemicals;
- investigate the impact of primary and secondary emissions on the spatiotemporal trends of organic chemicals;
- assess the relationship of these concentrations in different environmental compartments;
- assess the potential adverse effects of realistic mixtures of organic chemicals along environmental gradients;
- quantify the relative contribution of organic chemicals to the phytoplankton growth dynamics.

This research is described in five main chapters (Fig. 1.7). The study regions for each chapter are plotted in Figure 1.8.

1.6 Thesis outline in relation to research objectives and hypotheses

1.6.1 Spatiotemporal trends

In the late 1970s, the production and use of PCBs was stopped in Western Europe and North America (Box 1.2; Breivik et al., 2007). As PCBs are persistent substances, we investigated whether the ban has resulted in decreasing environmental concentrations. Even in intensively monitored ecosystems such as the southern part of the North Sea environmental concentrations of organic chemicals are relatively scarce. Incomplete datasets thus hamper the establishment of robust trends. Therefore, in **chapter II** we applied additive modelling to derive spatiotemporal trends of PCB concentrations collected in the sediment of the Belgian Coastal Zone (BCZ) and the Western Scheldt estuary. Based on literature findings in similar coastal-estuarine regions (Webster et al., 2011; Hong et al., 2010), we hypothesize that PCB concentrations in the sediment of the BCZ are decreasing (due to decreasing primary emissions). A second hypothesis is that concentrations in the Western Scheldt estuary are higher than those in open sea. Finally, it is expected that spatiotemporal trends of PCB concentrations in the Western Scheldt estuary are affected by industrial activities in the harbor of Antwerp (secondary emissions from historically polluted sediment).

1.6.2 Partitioning of organic chemicals between environmental compartments

Due to their hydrophobicity, organic contaminants have a relatively high affinity to adsorb to organic material, to settle in sediments and to accumulate in fatty tissue. Organic contaminants partition between different environmental compartments (air, water, sediment and biota) and based on the corresponding partitioning coefficients, their concentrations in these compartments are related. Hence, in **chapter III** we used additive models to infer spatiotemporal trends of PCB concentrations in the North Sea and Celtic Sea in sediments and biota. We expect to observe decreasing PCB time trends in sediments and mussels according to the general trends reported in literature. Based on the biological pump theory as described in Dachs et al. (1999) and Nizzetto et al. (2012) we hypothesize that PCB concentrations in the different environmental compartments are related.

1.6.3 Environmental risk assessment

During the past two decades, concentrations of organic contaminants in the marine environment have generally decreased (Law et al., 2010). However, it is not clear whether current concentrations still pose a risk to marine ecosystems. To assess this environmental risk, in **chapter IV** we compared spatiotemporal trends (inferred in chapter III) with quality threshold concentrations that are commonly used in regulatory/legislative contexts (e.g. EAC). Despite the decreasing trends in both environmental compartments (chapter III) considered, we expect that PCB sediment exceed the quality thresholds while PCB concentrations in mussels are below the quality criteria.

1.6.4 Ecotoxicological effect of a realistic chemical mixture

Comparing concentrations of chemicals with quality thresholds (as in chapter IV) only suggest a potential ecological risk and if risk quotients exceed the value of 1, additional assessments are recommended. Considering the results obtained in chapter IV, in **chapter V** we performed some additional experimental studies and exposed a marine diatom to a realistic mixture of organic contaminants in an algal growth inhibition test. We performed the algal growth inhibition test under different environmental conditions (nutrients, temperature and light intensities). The aim of this design, i.e. environmental conditions x POP stress was to assess the contribution of the former variables to the growth dynamics of the marine diatom relatively to that of the potential adverse effects of organic chemicals. Echeveste et al. (2010) reported no ecotoxicological effects of exposure to realistic mixtures (and concentrations) of commonly occurring marine micro-contaminants. Based on these findings, it was hypothesized that a realistic mixture of organic chemicals close to environmental concentrations did not hamper the growth of the marine diatom that was tested.

1.6.5 Relative contribution of organic chemicals to phytoplankton growth

Although the methodology used in chapter V is a standard way to assess toxicity of chemicals, the relevance of using just one algal species to represent ecotoxicological effects of an entire phytoplankton community may not be environmentally realistic. Therefore, in **chapter VI**, we used an ecosystem model to assess the potential adverse effects of organic contaminants on the total primary production. We implemented an ecosystem model on two decades of monitoring data collected along the Belgian coast and in the Kattegat. As in

chapter V, we estimated the contribution of the organic contaminants relative to the contribution of naturally changing environmental variables. Our hypothesis here was similar to that formulated in chapter V, i.e. that organic chemicals did not hamper the marine primary production.

1.6.6 Conclusion

Chapter VII reviews the results obtained in this research, summarizes the conclusions and presents some suggestions for future research.

Questions addressed by this dissertation;

- Chapter II: How did PCB concentrations in sediments change in Belgian marine waters and in the Western Scheldt estuary? Are those spatiotemporal trends of PCB concentrations driven by primary emissions or secondary emissions?
- Chapter III: How did concentrations of organic contaminants in sediment and biota change in the North Sea and the Celtic Sea between 1979 and 2012? Are those changes interrelated between different environmental compartments and linked to the seasonal phytoplankton cycle?
- Chapter IV: Following the environmental quality thresholds, how did the potential risk of organic contaminants change in the North Sea and the Celtic Sea between 1979 and 2012?
- Chapter V: Does a realistic mixture of organic contaminants alter the growth dynamics of a marine diatom under laboratory conditions? What is the relative contribution of organic chemicals, nutrients, light energy and temperature to the growth dynamics?
- Chapter VI: Is the marine primary production affected by the multitude of organic contaminants present in the marine environment? What is the relative contribution of organic chemicals, nutrients, light energy and temperature to the growth dynamics of marine primary production based on long-term field data?

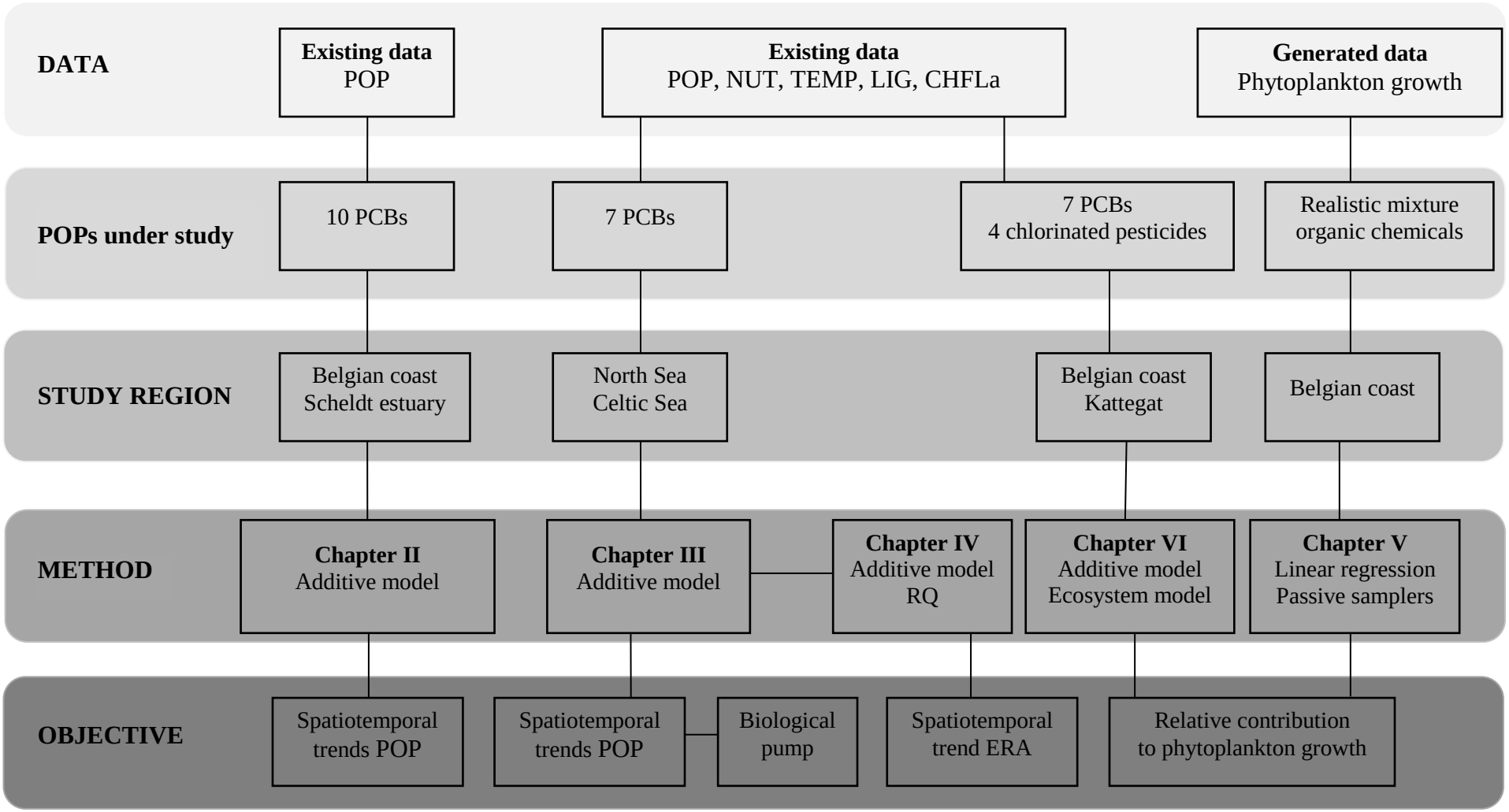


Figure 1.7 *Schematic overview of the data used, the persistent organic pollutants studied, the study region (see also Fig. 1.8), the methodology and the research objectives for each chapter included in this dissertation.*

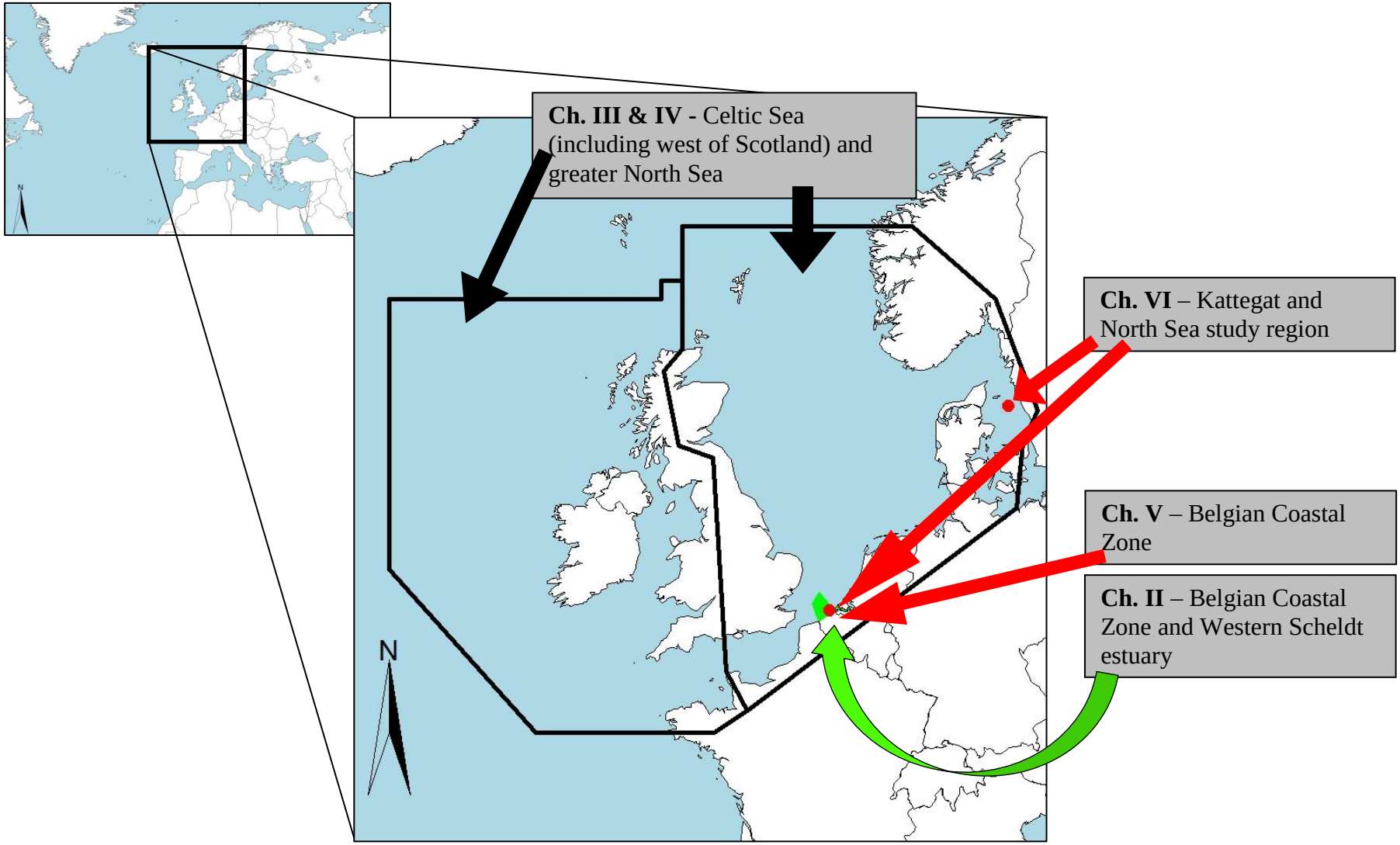


Figure 1.8 *Map of the study region for each chapter included in this dissertation.*

2

Spatiotemporal trends of polychlorinated biphenyls in Belgian coastal and estuarine sediments

Redrafted from:
Gert Everaert, Frederik De Laender, Klaas Deneudt, Patrick Roose, Jan Mees, Peter L.M. Goethals, Colin R. Janssen (2014). Additive modelling reveals spatiotemporal trends of POPs in marine sediments. Marine Pollution Bulletin. 79, 47-53.

Abstract

We developed generalized additive mixed models (GAMMs) to infer spatiotemporal trends of environmental PCB concentrations from an extensive dataset (n = 1219) of PCB concentrations measured between 1991 and 2010 in sediments of the Belgian Coastal Zone (BCZ) and the Western Scheldt estuary. A GAMM with time, geographical zone, periodicity and the organic carbon – water partition coefficient as covariates explained 49% of the variability in the log transformed PCB sediment concentrations. The time trends unraveled two to three-fold PCB concentration decreases in the BCZ during the last 20 years. However, in the Western Scheldt estuary, time trends were spatially heterogeneous and not significantly decreasing. These results demonstrate that international efforts to cut down emissions of PCBs have been effective to reduce concentrations in open water ecosystems like the BCZ but had little effect in the urbanized and industrialized area of the Scheldt estuary.

2.1 Introduction

The environmental fate and toxicity of persistent organic pollutants (POPs) are of global concern. POPs concentrate in fatty tissues and magnify in food chains due to their hydrophobic and lipophilic properties (Jones & de Voogt, 1999). Their persistence facilitates their transport to and impacts on regions far from their sources (Muir et al., 2000). International conventions, such as the United Nations Environment Programme (UNEP) Stockholm convention on POPs, aim for cross-border governance of this problem by controlling the primary POP sources and eliminating POP emissions globally by 2020 (UNEP, 2001). To quantify their spatiotemporal distribution and evaluate the success of regulations, monitoring activities intensified at the beginning of the 1990s (Lamon et al., 2009; Lohmann et al., 2012). In particular, spatiotemporal trend analyses of hazardous substances are stipulated as a key priority by the OSPAR commission and data collection frameworks such as the Coordinated Environmental Monitoring Programme (CEMP) were initiated (ICES, 2004). Polychlorinated biphenyls (PCBs) are often used as model compounds to study the environmental behaviour of POPs (Schuster et al., 2010; De Laender et al., 2010). PCB time trend studies performed in air (e.g. Schuster et al., 2010; Venier et al., 2012), seawater (e.g. Gioia et al., 2008), biota (e.g. Roose et al., 1998; De Laender et al., 2011; Weijs et al., 2012) and polyethylene pellets (e.g. Ryan et al., 2012) indicate decreasing PCB trends, suggesting legislative efforts have been successful (Lohmann et al., 2007). However, site-specific hotspots of PCBs (Wurl & Obbard, 2005; Gioia et al., 2008) have been detected, making it unsure if global PCB decreases are also occurring near densely populated industrialized sites (de Wit et al., 2006; Gedik et al., 2010; Van Ael et al., 2012) and in estuaries (Danis et al., 2004; Carroll et al., 2007; Webster et al., 2011).

The southern part of the North Sea and the Western Scheldt estuary are globally amongst the most intensively monitored marine and estuarine regions. Despite the high degree of industrialization and urbanization present in this region (Hesse, 2010), Breivik et al. (2007) has simulated decreasing congener-specific primary atmospheric PCB emissions between 1991 and 2010. However, the question is whether the decreasing atmospheric PCB concentrations are also reflected in other environmental compartments (e.g. water, sediment and biota). For instance, Berrojalbiz et al. (2011) found that different environmental compartments do not always follow identical trends. Additionally, we ask if different PCB congeners follow similar trends. In case of primary point source pollution, it is hypothesised that dissimilar trends will be observed per congener. However, under secondary diffuse source pollution, similar trends across congeners are expected.

In this paper we analyse one of the longest and most complete data sets of PCBs using a regression-based technique in order to compare the levels and time trends of marine and estuarine PCB sediment concentrations between and within the Belgian Coastal Zone (BCZ) and the Western Scheldt estuary. We used 1219 monthly averages of PCB sediment concentrations, representing ten PCBs measured in a twenty-year period. To test the robustness of the inferred time trends, we compared these trends to 614 independent monthly averages of PCB concentrations. Finally, in order to compare different environmental compartments, the PCB sediment time trends inferred in this research were compared to PCB emission estimates of Breivik et al. (2007).

2.2 Data and models

2.2.1 Data

Sediment concentrations of polychlorinated biphenyls (PCBs) in the sediment fraction smaller than 63 µm along the Belgian Coastal Zone (BCZ) and the Western Scheldt estuary were obtained from the online database of the Belgian Marine Data Centre (BMDC, http://www.mumm.ac.be/). The sediment fraction smaller than 63 µm was selected as the OSPAR guidelines for temporal trend monitoring recommend the determination of contaminants in the fine fraction of the sediment (ICES, 2012). Indeed, most samples were taken in the sediment fraction smaller than 63 µm (Table A.1; Fig. A.1). In total, 4297 PCB concentrations representing ten congeners were extracted from the BMDC. The study area was divided in eight zones: four in the Western Scheldt estuary and four at the BCZ (Fig. 2.1).

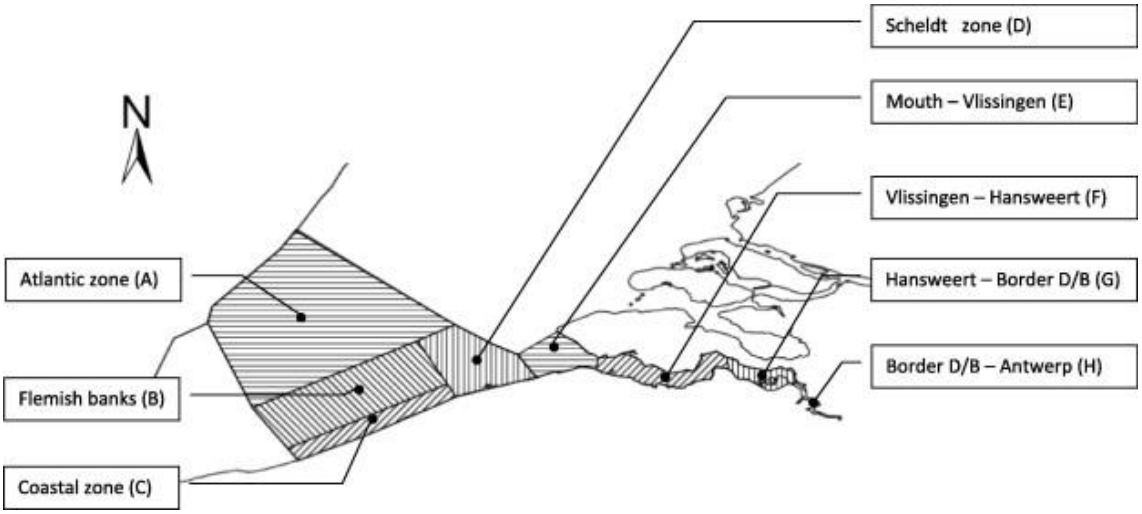


Figure 2.1 *Eight subzones delimited in our study area: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F) from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

Prior to the modelling, we detected three outliers by means of Cleveland dotplots (Table A.2) and removed these from the data. Furthermore, we discarded 376 values that were below the detection limit. The authors would like to refer to figures A.2 – A.3 in supportive information for the results of parallel model development including these values. Briefly, it was found that unwanted noise was added in the statistical analyses when including the detection limits. Next, the remaining 3921 sediment concentrations ($\mu g.g^{-1}$) were log10 transformed (Zuur et al., 2010) and, based on the reported latitude and longitude of the sampling location, assigned to one of the eight zones (Fig. 2.1). Monthly averaging of the reported sediment concentrations ($\mu g.g^{-1}$ on a dry weight basis) resulted in a dataset comprising of 1833 records representing monthly mean sediment concentrations of ten PCBs measured between 1991 and 2010. Two thirds of this dataset (n = 1219) was randomly selected for model fitting, the remaining one third (n = 614) was used to assess the trends we found (Tables A.3 – A.6). All data preprocessing steps were performed in R (R Development Core Team, 2010).

2.2.2 Statistical modelling

The overall objective of our research was to infer PCB time trends for the BCZ and the Scheldt estuary and to verify which PCB congeners show similar spatiotemporal gradients. To this end, additive modelling was selected; a nonparametric alternative to the more conventionally used generalized linear models (GLMs). Generalized additive models (GAMs) only differ from GLMs in that GAMs use nonparametric smooth functions of the explanatory variables whereas GLMs use products between the regression coefficients and the explanatory variable (Wood, 2006):

$$g^{-1}\left[E\left(Y_i|X_{ij}\right)\right] = \alpha + \sum_{j=1}^{k} f_j\left(X_{ij}\right) \qquad \text{(Eq. 2.1)}$$

where g specifies the link function between the expected value of Y_i (PCB concentration) and the explanatory variables X_{ij}, with i = 1 to n the number of the observations and j = 1 to n the number of explanatory variables. The smoother function $f_j(x_{ji})$ quantifies the effect of the j^{th} explanatory variable on Y_i and α represents the estimated regression coefficient. The number of knots of f_j represents the amount of smoothing applied to the data (Wood & Augustin, 2002). The value of k should be chosen to be large enough to allow sufficient degrees of freedom to represent the underlying relation, but small enough to maintain reasonable computational efficiency. A smoother with two knots is linear, has little variability and may be biased since there is only one piecewise function (Keele, 2008). Increasing the number of knots allows more flexibility, but may result in overfitting. The optimal number of knots depends on sample size and data pattern; for sample sizes above 100, five knots provide a good compromise between flexibility and overall fit (Keele et al., 2008). For smaller samples, below 30, the use of three knots is a good starting point. Keele (2008) report that four to five knots is appropriate for most applications. In our analysis, the maximum number of knots for the smoothing curves was fixed to four, but the actual degrees of freedom are controlled by the degree of penalization selected during the model fitting. All additive models were fitted using the mgcv package in R (R Development Core Team, 2010).

2.2.3 Model fitting

We considered three different model configurations to fit to the data, only differing in the covariates they contained (Table 2.1). All three models included time (as year and month), zone and periodicity as covariates. Time and zone (Fig. 2.2) were included because our objective was to quantify spatiotemporal trends. Periodicity was represented by a period τ and amplitude A, because an interannual repetitive fluctuation was observed during data exploration. In essence, the effect of periodicity is the same for two concentrations separated in time by a multiple of τ years, i.e. f(x + τ) = f(x). However, when the time interval is not a multiple of τ, periodicity affects two concentrations differently, depending on the amplitude. We used a cycling period of eight years as shorter or longer cycling periods proved to fit less well to the observed periodicity in the data (Fig. A.1). Apart from time, zone and periodicity, the first model (model 1) also included the chemical name of the PCB (termed "Substance") as a fourth covariate, allowing for PCB-specific time trends (Table 2.1). In the second model (model 2), the variable "Substance" (in model 1; Table 2.1) was replaced by the log10 organic

carbon – water partition coefficient (log K_{oc}; in model 2; Table 2.1). The log K_{oc} values were obtained from the freely available EPI Suite™ v4.10 application (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm). Due to the typical trade-off between model accuracy and general applicability (Guisan & Zimmerman, 2000), we expected model 2, including the descriptor log K_{oc}, to have a lower predictive power than model 1 (Table 2.1). Lastly, because modelling marine data can result in misleading predictions due to autocorrelation of the samples (Merckx et al., 2011), we tested if accounting for this autocorrelation resulted in a better model by comparing the Akaike Information Criterion (AIC) of model 2 to the AIC of a third model that explicitly included autocorrelation (model 3). The AIC rewards proximity to the data but penalizes model complexity. Lower AIC values indicate a better model. Because sampling occurred irregularly in time, a continuous correlation structure was selected (Table 2.1). The correlation coefficient (φ) of this structure was fitted to the data and its value was used to evaluate the strength of autocorrelation in the data. Positive φ values indicate that data at any particular point in time are positively related to preceding time points (Wood, 2006). The closer this value is to 1, the more autocorrelative the data are (Zuur et al., 2009).

Table 2.1 *Different modelling approaches used to infer time trends of the POP sediment concentrations. The predictors available are listed in columns, per modelling approach the significance of the predictors included is shown by means of its p-value.*

	model 1	**model 2**	**model 3**
Model formulation	C_{sed} ~ s(Year|Zone) +s(Periodicity) +Substance +Zone	C_{sed} ~ s(Year|Zone) +s(Periodicity) +s(log Koc) +Zone	C_{sed} ~ s(Year|Zone) +s(Periodicity) + s(log Koc) +Zone +CorCAR1
Predictors			
Time	$2.0 * 10^{-16}$ < p < $2.1 * 10^{-1}$	$2.0 * 10^{-16}$ < p < $3.2 * 10^{-1}$	$2.0 * 10^{-16}$ < p < $4.3 * 10^{-1}$
Zone	$< 2.0 * 10^{-16}$	$< 2.0 * 10^{-16}$	$< 2.0 * 10^{-16}$
Periodicity	$1.4 * 10^{-15}$	$2.2 * 10^{-9}$	$1.5 * 10^{-13}$
Substance	$< 2.0 * 10^{-16}$		
log Koc		$< 2.0 * 10^{-16}$	$< 2.0 * 10^{-16}$
CorCAR1			$\varphi = 0.14$
Predictive power			
R^2	0.69	0.47	0.47
AIC	556	1210	1169

2.2.4 Residual diagnostics and model robustness

When analyzing environmental data using a regression-based technique, it is particularly important to test the underlying assumptions (i.e. homogeneity and normality) based on the residuals of the fitted model (Zuur et al., 2009). Homogeneity was concluded if the variability

of the residuals was similar across the range of fitted values. Normality was assessed using graphical diagnostics such as the QQ plot (residuals should be on a straight line) and the histogram of the model residuals (a bell-shaped curve is expected; Wood, 2006; Zuur et al., 2009). The model configuration combining a low AIC and acceptable residual diagnostics (see Zuur et al., 2009) was selected as the optimal model configuration. The robustness of the model was assessed by applying it to the independent data (n = 614), i.e. data that were not used for model calibration. To this end, every PCB concentration in the independent data set was compared to the corresponding prediction.

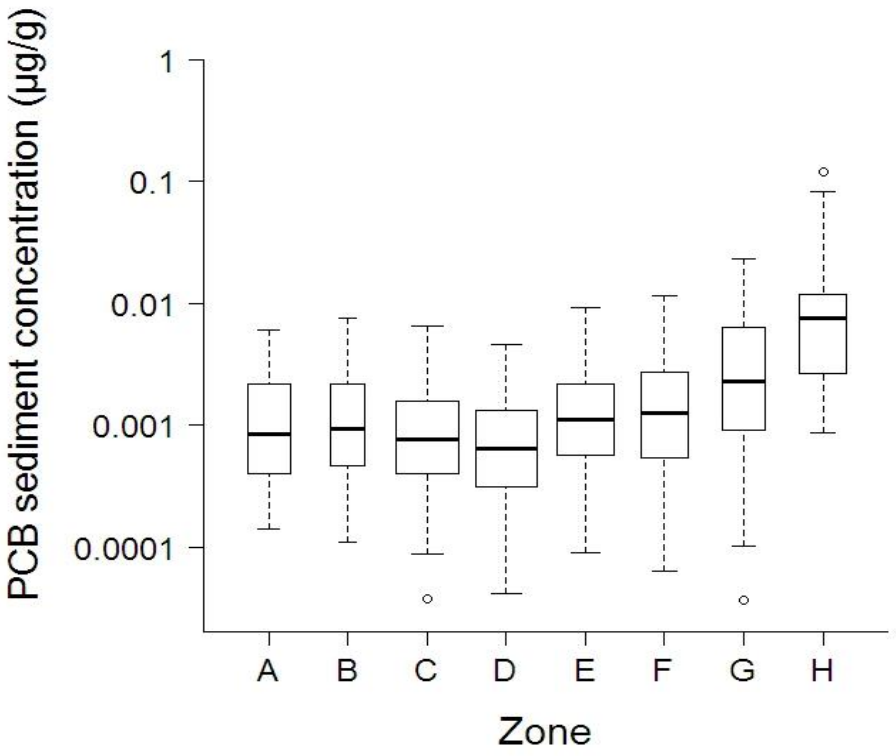


Figure 2.2 *Average observed PCB concentrations per zone between 1991 and 2010. The widths of the boxes are proportional to the square-roots of the number of observations per zone: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

2.2.5 Time trend interpretation using emission data

The time trends we inferred between 1991 and 2010 were compared to the PCB emission trends for the same period published in Breivik et al. (2007). The latter dataset contains yearly global congener-specific atmospheric PCB emission predictions for a 1° x 1° grid between 1930 and 2100. The PCB emission estimates are based on population density within the grid square. In accordance to Schuster et al. (2010), the grid square at the emission data were chosen closest to the geographical position of the studied zone. The PCB time trends in the BCZ and the Scheldt estuary were compared to the PCB emission trends for grid 51.5° x 2.5 and grid 51.5° x 3.5°, respectively. The nonparametric Spearman correlation coefficient was used and assessed at a 0.01 level of significance.

2.3 Results and Discussion

2.3.1 Model selection

Model 1, including one intercept and one time trend per polychlorinated biphenyl (PCB), indicated that the periodic oscillation and zone-specific temporal averages were significant covariates of PCB sediment concentrations (Table 2.1). The congener-specific intercepts of model 1 increased with the log10 organic carbon – water partition coefficient (log K_{oc}; Fig. 2.3), most notably for PCBs with log K_{oc} smaller than 5. For more hydrophobic substances (log K_{oc} > 5), this increase was less evident. High lipophilicity often reflects large molecule sizes, causing a decrease in the chemical's potential to adhere to environmental matrices due to steric hindrance (Fisk et al., 2001; Jonker & Koelmans, 2002; Hung et al., 2010).

Although, the fit of model 2, including the log K_{oc} of the PCB as a covariate, was lower than the fit of model 1 (Table 2.1), the time trends were similar, i.e. PCB concentrations decreased in the Belgian Coastal Zone (BCZ) and were relatively stable in the estuary. The fact that the log K_{oc} could successfully replace the "Substance" covariate indicates that the ten PCB congeners studied followed the same trend. As such, the log K_{oc} can be used to understand the behaviour of those ten congeners. Since the ten congeners followed the same trend, no primary point sources are expected in the BCZ and Scheldt estuary as in case of point sources we would expect congener-specific trends.

Model 3, including an autocorrelation structure ($\varphi = 0.14$), indicated that the data were only slightly auto-correlated. However, the AIC of model 3 (AIC = 1169) was lower than that of model 2 (AIC = 1210), indicating the data were explained better when including the autocorrelation structure (Table 2.1). The residuals of model 3 follow a normal distribution (Fig. A.5) and when plotting the residuals versus the covariates no patterns were observed. This indicates that the normality and homogeneity assumptions, and therefore the derived p-values, were valid. Testing of model 3 against independent data demonstrated that the model was capable of capturing zonal differences in PCB sediment concentrations (Fig. 2.4). Except for the lowest and highest sediment concentrations (which were slightly over- and underestimated, respectively), the time trends we found based on model 3 deviate less than one order of magnitude of those reflected by the independent data set, showing that the inferred time trends are robust within the study region (Fig. 2.4). Considering the effect of analytical variation between laboratories on measured PCB concentrations (Wells & de Boer, 2006) and the environmental variation of PCB concentrations (Lohmann et al., 2012), which both may be as high as two to three order of magnitude, the accuracy of the inferred time trends was considered adequate. The overprediction of low observed values (left lower corner in Fig. 2.4) and underprediction of high observed values (upper right corner in Fig. 2.4) probably relates to site-specific properties of the sampling locations. A possible explanation for the overprediction of the low observed values is that close to the harbour of Antwerp, few relatively "clean" sites were monitored. However, based on the additive model, we draw the general conclusion that the zone closest to the harbour of Antwerp is most polluted (Fig. 2.2). Hence, all samples collected in this zone are considered as polluted, which may not be the case in some locations (leading to the overprediction; Fig. 2.4). A similar explanation holds for the underprediction of some of the high observed values (Fig. 2.4).

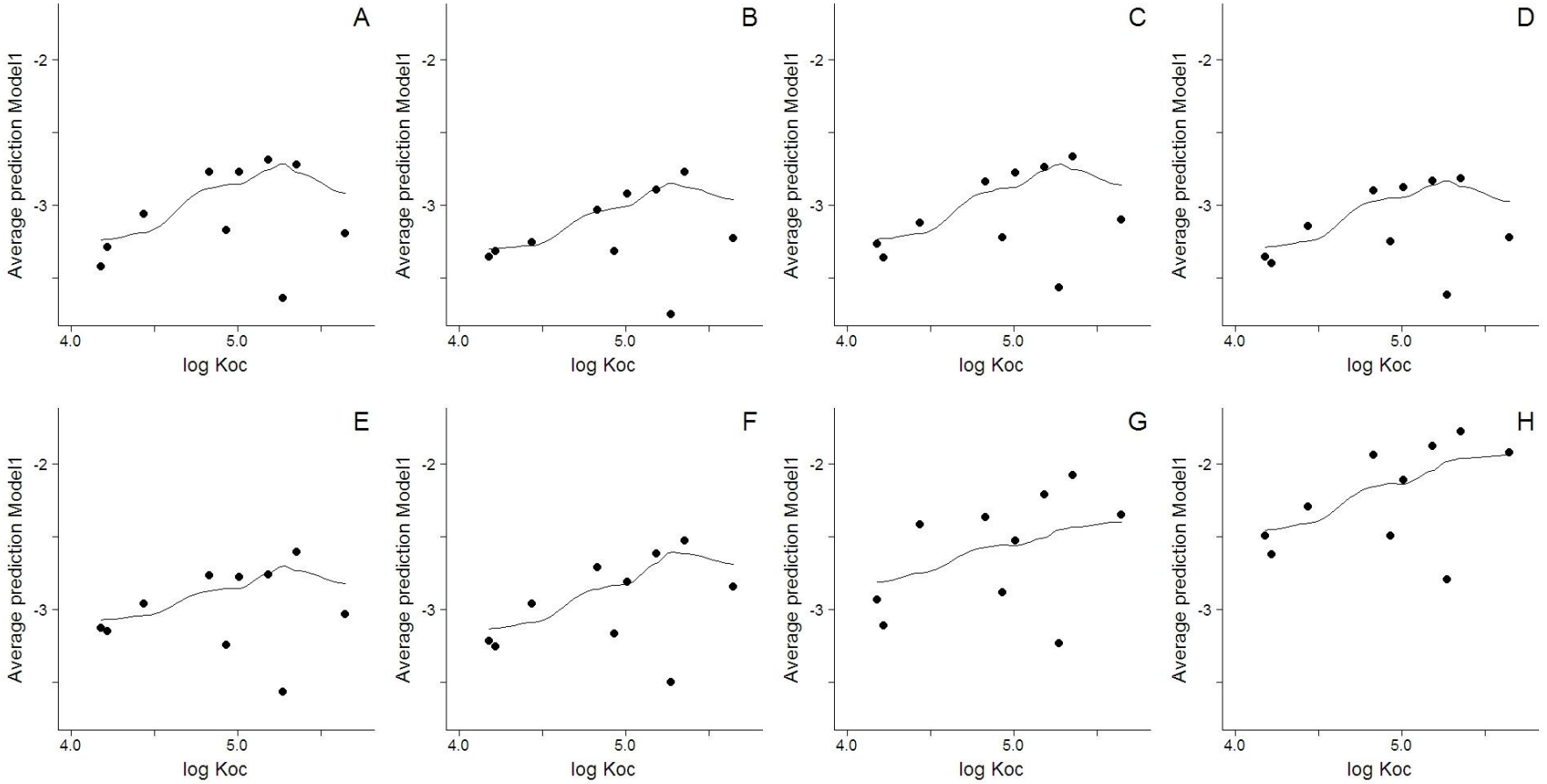


Figure 2.3 *Per PCB, the intercept of the additive model was extracted and plotted against its log10 organic carbon – water partition coefficient (log K_{oc}) by zone: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H). The lower the log K_{oc}, the lower the intercept of the model. However, at the highest log K_{oc} values the log K_{oc} effect is limited. In one substance class, log K_{oc} – intercept relations are similar for the different zones. Therefore, we concluded that is valid to replace the grouping variable "Substance" by the numerical variable log K_{oc}.*

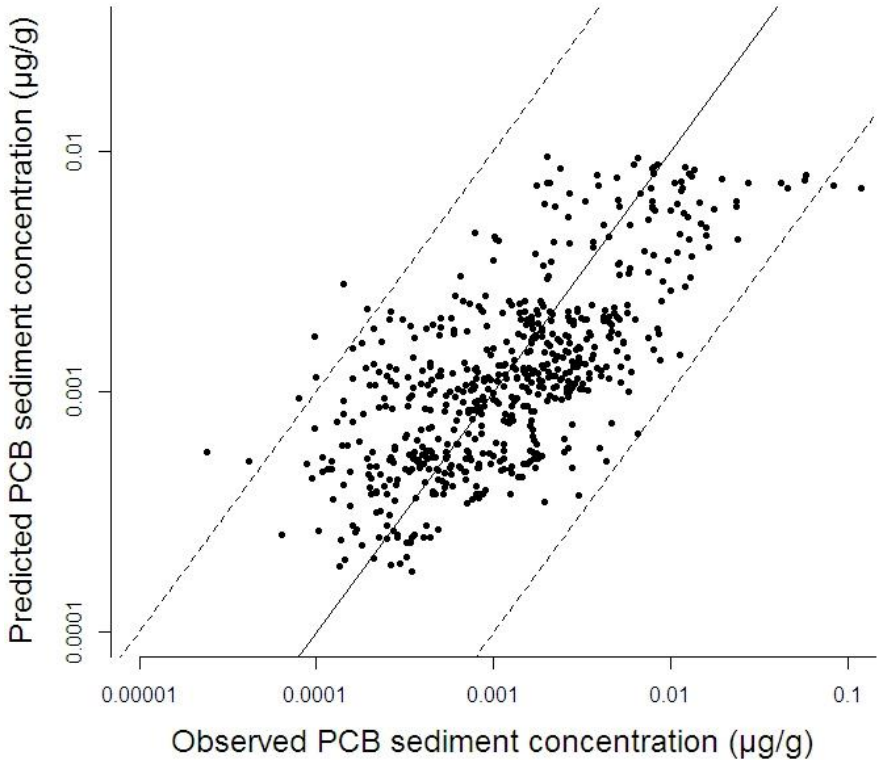


Figure 2.4 *Application of model 3 (Table 2.1) on the independent data set (n=614). The estimated PCB sediment concentrations are compared with the observed concentrations. The solid line represents the first bisector and the dotted lines represent one order of magnitude deviance.*

2.3.2 PCB time trends and levels

The time trends indicated decreasing PCB sediment concentrations in the BCZ (Fig. 2.4). In 1991, PCB concentrations (on a dry weight basis) were on average two to three-fold higher than today and currently vary around 0.56 ng.g^{-1} (0.19 ng.g^{-1} (10th percentile) – 1.03 ng.g^{-1} (90th percentile)). The decreasing PCB sediment concentrations in the BCZ corroborate with what has been described for other coastal and estuarine regions (e.g. Konat & Kowalewska, 2001; Johannessen et al., 2008; Hong et al., 2010) and other environmental compartments (e.g. Roose et al., 1998; Schuster et al., 2010; De Laender et al., 2011).

In contrast with the time trends for the BCZ, PCB time trends in the Scheldt estuary differed among zones. In general, PCB concentrations in the Scheldt estuary remained stable between 1991 (3.08 ng.g^{-1} (mean); 0.69 ng.g^{-1} (10th percentile) – 8.15 ng.g^{-1} (90th percentile)) and 2010 (2.79 ng.g^{-1} (mean); 0.26 ng.g^{-1} (10th percentile) – 19.56 ng.g^{-1} (90th percentile)). This was reflected by the smoothers for zone F, G and H. In zone F, PCB concentrations did not change with time (0.87 ng.g^{-1} (mean); 0.71 ng.g^{-1} (10th percentile) – 3.05 ng.g^{-1} (90th percentile) in 1991 versus 1.59 ng.g^{-1} (mean); 0.70 ng.g^{-1} (10th percentile) – 1.96 ng.g^{-1} (90th percentile) in 2009). In the zone between Hansweert and the Dutch-Belgian border (zone G), PCB concentrations decreased up to the year 2000, but then increased again. In the zone closest to the port of Antwerp (zone H) the PCB concentrations remained relatively unchanged (7.22 ng.g^{-1} (mean); 3.44 ng.g^{-1} (10th percentile) – 14.75 ng.g^{-1} (90th percentile) in 1991 versus 6.78 ng.g^{-1} (mean); 2.36 ng.g^{-1} (10th percentile) – 10.11 ng.g^{-1} (90th percentile) in 2006; Fig. 2.5).

The inferred time trend at the mouth of the river Scheldt (zone E, neighboring the BCZ) followed the same pattern as the time trends found in the BCZ. The PCB sediment concentrations in zone E decreased threefold between 1992 (0.85 $ng.g^{-1}$ (mean); 0.53 $ng.g^{-1}$ (10^{th} percentile) – 2.28 $ng.g^{-1}$ (90^{th} percentile)) and 2010 (0.26 $ng.g^{-1}$ (mean); 0.19 $ng.g^{-1}$ (10^{th} percentile) – 0.80 $ng.g^{-1}$ (90^{th} percentile)).
The PCB sediment concentrations in the Western Scheldt estuary always exceeded the concentrations in the BCZ (Fig. 2.2). This has already been reported for PCBs (Danis et al., 2004) and for dissolved metal concentrations (Baeyens et al., 1998). In 2004, Voorspoels et al. (2004) reported an inverse relation between the POP concentrations in benthic species and the distance between sampling points and the port of Antwerp. Also in Korea, Hong et al. (2010) found a positive correlation between the distribution and levels of organochlorine contaminants and shipping and industrial activities. More recently, Webster et al. (2011) concluded that the PCB sediment concentrations in the Clyde estuary (Scotland) exceeded those of the open sea and related this to the fact that the estuary was subject to (in)direct inputs.

Although the PCB congeners studied were identical (i.e. same breakdown rates) for both regions, dissimilar time trends were observed in the BCZ and the Scheldt estuary (Fig. 2.5). This suggests the presence of PCB inputs in the estuary, which can be indirect from other environmental compartments based on the PCB partitioning characteristics or direct via the industrial activities in the port of Antwerp (e.g. commodity-throughput, oil refinery, petro chemistry). Although the port of Antwerp is the largest chemical cluster in Europe (Hesse, 2010), direct inputs via point sources are unlikely as the PCB congeners studied followed identical trends (see 2.3.1). This suggests that mainly indirect diffuse inputs are the main sources of PCBs. However, Breivik et al. (2007) simulated decreasing atmospheric PCB concentrations and we found relatively stable PCB sediment concentrations in the Scheldt estuary. This was reflected in the non-significant correlation coefficients between the PCB sediment time trends inferred and the emission estimates of Breivik et al. (2007; Table A.7). This indicates that other PCB sources than the atmospheric deposition contributed to those stable PCB levels. The latter can be explained by the dredging activities, which are permanently carried out to maintain the maritime fairway to the harbour of Antwerp (Plancke et al., 2008). These activities consist of dredging natural sills in the fairway and relocating the dredged sediment (Pynaert & Speleers, 2004). Polluted sediment has been previously identified to control the concentrations in the other environmental compartments (Lohmann et al., 2007; Ishaq et al., 2009). Also, Schulzbull et al. (1995) observed surprisingly high PCB concentrations in the southern part of the Baltic Sea and related this the relocation PCB polluted sediment. In the Indiana harbour and ship canal in Lake Michigan dredging resulted in release and redistribution of PCBs in the environmental compartments (Martinez et al., 2010). Overall, due to the myriad of activities going on, the proximity of urbanized and/or industrialized areas may lead to higher PCB levels and different PCB trends compared to the open sea (de Wit et al., 2006; Choi et al., 2011; Yang et al., 2011). The PCB inputs are mainly from diffuse secondary origin and therefore the regulatory efforts, which have successfully reduced the preliminary PCB sources in many ecosystems, should now focus on the secondary pollution sources.

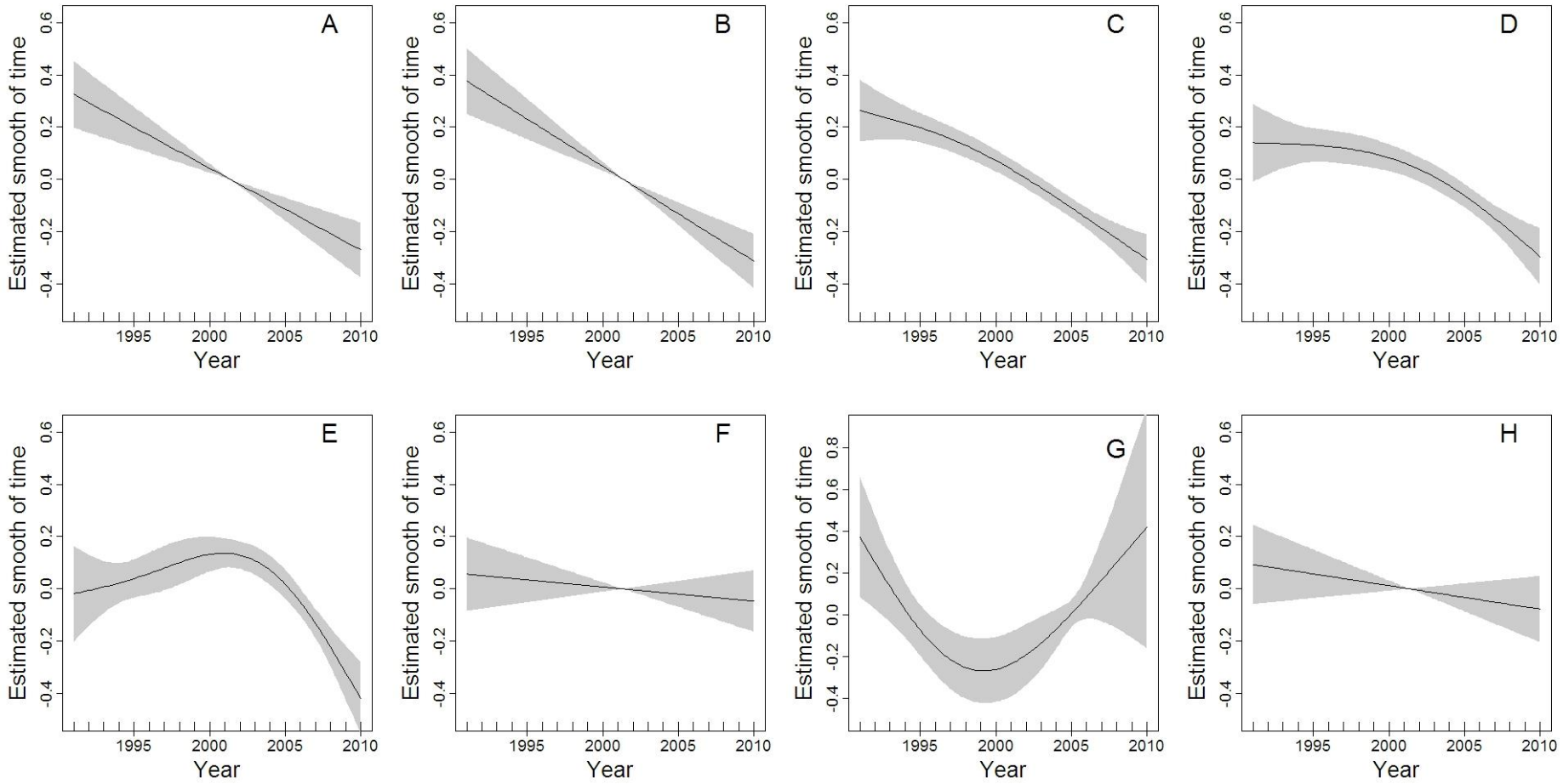


Figure 2.5 *Zone dependent time smoothers for the model 3: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

Superimposed on the spatiotemporal effect, we found an interannual oscillation of the sediment PCB concentrations (Fig. 2.6). After attempting various oscillation periods (5 to 11 years), the best fit was obtained with an oscillation period of eight years (Fig. A.1), which is similar to the period of the North Atlantic Oscillation (NAO; Hurrell & Van Loon, 1997). The NAO influences temperature and precipitation across the North Atlantic basin, exerts a major impact on marine ecosystems (Hurrell, 1995) and has been shown to influence PCB concentrations in the Canadian Arctic atmosphere (Hung et al., 2005). The mechanism allegedly explaining the relationship between the NAO and the distribution of POPs suggests that positive phases of the NAO (i.e. high temperatures) enhance the volatilization of POPs and thus influence their environmental behaviour (Pelley, 2004). In 2010, Ma et al. concluded that the interannual fluctuations in POP time trends are related to climate variability caused by the NAO and the El Nino-Southern Oscillation (ENSO). Also in the freshwater environment, POP concentrations measured around the Great Lakes during the 1990s showed a strong association with climatic oscillation patterns (Ma et al., 2004).

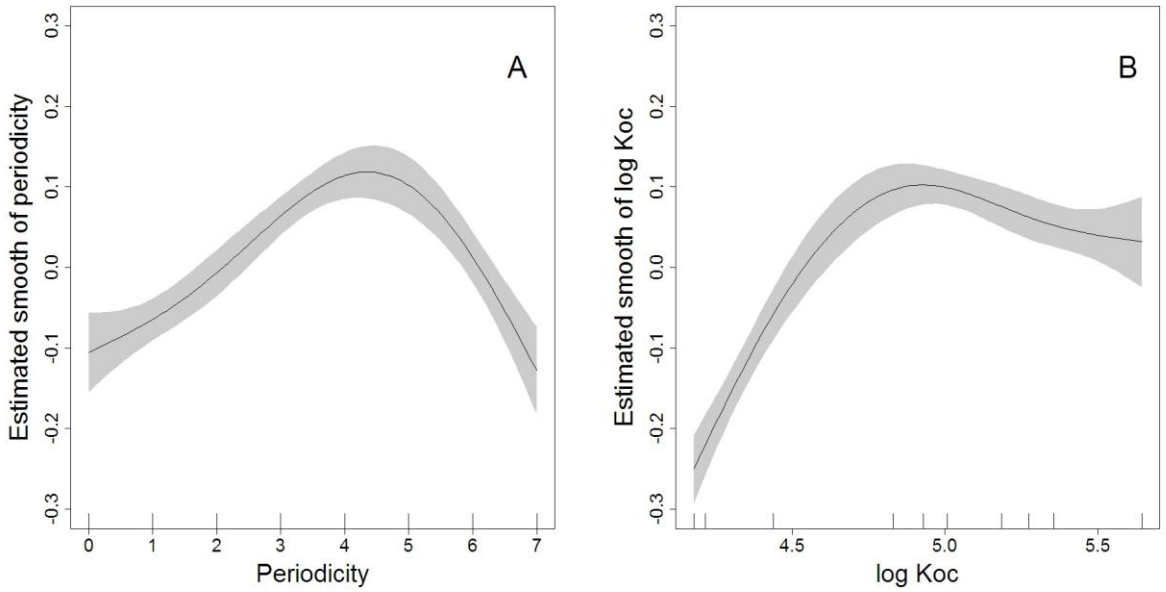


Figure 2.6 *Smoothers for the periodicity (A) and the log10 organic carbon - water partition coefficient (log K_{oc}) (B) of model 3. The small vertical lines above the x-axis in panel B indicate the log K_{oc} values present in the dataset.*

2.4 Conclusion

Environment legislation (e.g. OSPAR convention) successfully reduced PCB concentrations in the BCZ. The ten PCB congeners that were considered in the present study followed the same temporal trends, suggesting that no point sources are present in our study area for these congeners. The main PCB input is from indirect origin from historically polluted sites, preventing decreasing PCB concentrations in sediments of highly industrialized and urbanized estuaries.

3

Multidecadal and seasonal trends of polychlorinated biphenyls in the North Sea and Celtic Sea: the biological pump

Redrafted from:
Gert Everaert, Frederik De Laender, Peter L.M. Goethals, Colin R. Janssen. First evidence that multidecadal field data support intimate links between phytoplankton dynamics and PCB concentrations. Environmental Science and Technology. Under review.

Abstract

We analysed multidecadal field observations (1975–2012) in the North Sea and Celtic Sea with additive models to infer spatiotemporal concentration trends of chlorophyll a and sediment organic carbon, and of polychlorinated biphenyls (PCBs) concentrations in mussels and in sediments. By doing so, we separated long-term changes in PCB concentrations from seasonal variability. Using the inferred seasonal variability, we demonstrated a strong link between the chlorophyll a concentrations, the organic carbon concentrations and the PCB concentrations in the sediment and the blue mussel (*Mytilus edulis*). We found that the timing of phytoplankton blooms in spring and autumn corresponds to the annual maxima of the organic carbon content and the PCB concentrations in sediments. These results demonstrate the role of seasonal phytoplankton dynamics in the environmental fate of PCBs at large spatiotemporal scales.

3.1 Introduction

The marine environment is a sink for many chemical substances (Froescheis et al., 2000; Lohmann et al., 2006). Persistent organic pollutants (POPs) for example are, due to their low solubility in sea water and their persistence and tendency to sorb to organic matter, typically found at high concentrations in marine sediments and biota (Letcher et al., 2010; Sobek et al., 2014). Biogeochemical processes play an important role in the environmental fate of POPs (Riget et al., 2010; Nizzeto et al., 2012). One of the underlying mechanisms in the life cycle of POPs is their uptake by marine phytoplankton (Gasol et al., 1997) and the subsequent transfer of the plankton-bound POPs to deeper water and sediment (Lohmann et al., 2006; Dachs et al., 1999; Jaward et al., 2004; Turchetto et al., 2012). When phytoplankton blooms, dissolved-phase POP concentrations in surface water are depleted (Skoglund et al., 1996), which in turn promotes the air-to-water flux of lipophilic POPs (Nizzetto et al., 2012; Galban-Malagon et al., 2012). The role of this biogeochemical process, known as the biological pump (Nizzetto et al., 2012; Dachs et al., 1999), in the sequestration of lipophilic POPs was recently examined in the Atlantic and Arctic oceans (Jaward et al., 2004; Galban-Malagon et al., 2012) and in the Mediterranean sea (Berrojalbiz et al., 2011). In these studies, field POP concentrations were simultaneously collected in the different environmental compartments and provided valuable information on the partitioning processes of POPs (Galban-Malagon et al., 2012). As equilibrium partitioning between environmental compartments governs the environmental fate of POPs (Schenker et al., 2014), seasonal changes in phytoplankton biomass result in seasonally variable POP concentrations (Jurado et al., 2004).

Although well-described in theory, empirical evidence of the working of the biological pump is limited (Nizzetto et al., 2012). Field study data are often collected in one growth season along the navigational route of a research vessel (Galban-Malagon et al., 2012; Berrojalbiz et al., 2011). As such, it is unclear whether spatial and temporal variability due to pollution gradients from coastal water to open ocean (Gomez-Guttierrez et al., 2007) and interannual changes in POP concentrations (Everaert et al., 2014; Konat and Kowalewska, 2001) complicate the detection of the biological pump, if it is present. More specifically, at present, the coupling between phytoplankton and POP dynamics has not been demonstrated on a multidecadal time scale and the spatial scale of a regional sea.

The present paper aims to delineate seasonal and interannual variability of POP concentrations in sediment and biota as a result of changes in phytoplankton biomass. We examined whether the biological-pump-induced variable POP concentrations are inferable from field data collected in heterogeneous and dynamic European regional seas. To this end, we investigated an extensive set of data reporting interannual, seasonal and spatial variation of PCB concentrations in sediment (n = 2,185; 1990-2012) and in mussels (n = 4,116; 1979-2012), and of chlorophyll a concentrations (n = 703; 1975-2012) and sediment organic carbon content (n = 224; 1991-2012) in the greater North Sea (including the Kattegat) and the Celtic Sea (including the west of Scotland). Using additive modeling we separated long-term interannual changes, seasonal changes and spatial variability of the PCB concentrations. To examine the role of phytoplankton dynamics in the environmental fate of PCBs, we statistically compared the inferred spatiotemporal trends of PCB concentrations in sediment

and in mussels with those of sediment organic carbon and chlorophyll a concentrations. The novelty of the study lies in using large-scale existing time series to disentangle interannual from seasonal variations and spatial differences of PCB concentrations. As PCBs have a high affinity to stick to organic material, to settle in sediments and to accumulate in fatty tissues (Jones and de Voogt, 1999) we hypothesize that the PCB concentrations between the different environmental compartments are interrelated even though data were collected at large spatiotemporal scales.

3.2 Materials and methods

3.2.1 Data

Data were obtained from the International Council for the Exploration of the Sea (ICES, http://www.ices.dk/). The polychlorinated biphenyl (PCB) data included concentrations of seven congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) which were measured in sediment (< 2000 µm) and in the soft tissue of the blue mussel (*Mytilus edulis*). In this chapter we selected the PCB concentrations that were measured in the sediment fraction smaller than < 2000 µm as the final aim was to compare the spatiotemporal trends with the environmental assessment criteria (EAC) (see chapter IV). The main reason to do so is that the EAC have been defined without regard to sediment characteristics (ICES, 2012) and therefore, despite the fact that the finer sediments (e.g. fraction smeller than 63 µm as in chapter II) will have higher contaminant concentrations, one needs to analyse total sediments in order to assess sediment conditions (ICES, 2012). Chlorophyll a concentrations, used as a proxy for phytoplankton biomass (Ryther and Yentsch, 1957), were measured in the upper 3 m of the pelagic zone. Sediment organic carbon content and PCB concentrations were measured in the top layer of the sediment (< 0.02 m). PCB concentrations were converted to µg.kg dry sediment^{-1} (for sediment data) and µg.kg dry tissue^{-1} (for mussel data). We assumed a water content in wet sediment of 90% (Zaborska et al., 2008) and a 5% fat and 27% dry matter content in mussel tissue (Jauffrais et al., 2012). Only data for the greater North Sea (including Kattegat, further referred to as "North Sea") and the Celtic sea (including the west of Scotland, further referred to as "Celtic Sea") were considered and were selected using the ICES delineation of both ecoregions.

Prior to the modelling, we discarded concentrations that were below the detection limit and the remaining data were log10 transformed (Zuur et al., 2010). Based on reported latitudes and longitudes, we assigned the data to the corresponding seas and we calculated the distances to the nearest coastline for each sampling location. Next, we averaged PCB concentrations for each unique combination of year (1979-2012), month (1-12), congener (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180), matrix (sediment and mussel) and sea (North Sea and Celtic Sea). Averaging was done because covariates must have unique values within groups for CorCAR1 objects (used to take autocorrelation into account, see further, Zuur et al., 2009). Sediment organic carbon and chlorophyll a concentrations were averaged for each year (1975-2012), month (1-12) and sea (North Sea and the Celtic Sea). This procedure resulted in four sets of data: chlorophyll a concentrations (n = 703), sediment organic carbon contents (n = 224) and PCB concentrations in sediment (n

= 2,185) and mussels (n = 4,116). Finally, we randomly subdivided each of these datasets in a training set (2/3 of the data) and a validation set (1/3 of the data). In Tables B.1-B.3 we list, for each variable, the different steps taken and the amount of data that were retained after each step. The spatial distribution of the selected data is shown in Figures B.1-B.4. In addition to the PCB concentrations in the sediment and mussels, we also obtained sea water concentrations of congener 28 (PCB28) in the Celtic Sea and North Sea from the ICES data repository (http://www.ices.dk/). In total, 151 concentrations of PCB28 measured in the water layer between 2005 and 2011 were retained after an objective data selection (Table B.4).

3.2.2 Model

We applied an additive model (AM) to infer spatiotemporal trends for the PCB concentrations in sediment and mussels, sediment organic carbon content and chlorophyll a concentrations. Hence, the following model was applied:

$$g^{-1}\left[E\left(Y_i|X_{ij}\right)\right] = \alpha + \sum_{j=1}^{k} f_j\left(X_{ij}\right) \quad \text{(Eq. 3.1)}$$

where g specifies the link function between the expected value of Y_i (response variable) and the covariates X_{ij}, with i = 1 to n the number of the observations and j = 1 to n the number of covariates. The smoother function $f_j(x_{ji})$ quantifies the effect of the j^{th} covariate on Y_i and α represents the estimated regression coefficient. The number of knots of f_j represents the amount of smoothing applied to the data (Wood and Augustin, 2002). A smoother with two knots is linear, has little variability and may be biased since there is only one piecewise function. Increasing the number of knots allows more flexibility, but may result in overfitting. Keele (2008) reports that four to five knots is appropriate for most applications as it is a good compromise between flexibility and overall fit. In our analysis, the number of knots for the smoothing curves was fixed to five. Additive models were fitted using the *mgcv* package in R (R Development Core Team R, 2010).

To infer spatiotemporal trends of PCB concentrations in sediment and mussels we considered four different model configurations, only differing in the covariates incorporated (Table 3.1). All four configurations included time of sampling (as year and month), sampling location (name of the sea) and the congener name as covariates (configurations 1, 2, 3 and 4). A smoother for the distance of the sampling location to the coast was included in configurations 2, 3 and 4 (Table 3.1). We allowed a flexible residual spread for each month using a variance structure (Zuur et al., 2009) in configurations 3 and 4 (VarIdent; Table 3.1) and in configuration 4 we tested for autocorrelation between sequential observations using a continuous correlation structure (Zuur et al., 2009; CorCAR1; Table 3.1).

To infer trends of the sediment organic carbon content and the chlorophyll a concentrations similar model configurations were tested as used for the PCB concentrations. The main difference is that we did not include the name of the congener and the smoother for distance to the coast (except in configuration 2, but no convergence was found) (Table 3.2). The Akaike information criterion (AIC) and the model residuals (testing the homogeneity and

normality assumption) were used to assess the model fit (Zuur et al., 2009). The AIC rewards proximity to the data but penalizes model complexity. Lower AIC values indicate a better model (Zuur et al., 2009). To test the robustness of the selected model it was applied to independent data and predictions were plotted against observations to visualize model fit. Apart from AIC we also reported the adjusted r-squared value to quantify the proportion of variance explained.

3.2.3 Correlations

To determine the associations between the spatiotemporal trends of PCB concentrations in sediment and mussels, sediment organic carbon content and chlorophyll a concentrations, a nonparametric Spearman correlation test was used (with $\alpha = 0.05$). To examine the possible seasonal relationship between these variables, for each sea and for each variable, a one-season-long time trend was used.

Table 3.1 *Four different model configurations, one in each column, were tested to infer spatiotemporal trends of the polychlorinated biphenyl concentrations ([PCB]) in sediment and mussels in the North Sea and Celtic Sea. Models were assessed using the Akaike information criterion (AIC).*

Model configuration	[PCB] ~ s(Year|Sea) +s(Month) +Congener +Sea	[PCB] ~ s(Year|Sea) +s(Month) +s(Distance) +Congener +Sea	[PCB] ~ s(Year|Sea) +s(Month) +s(Distance) +Congener +Sea Varident	[PCB] ~ s(Year|Sea) +s(Month) +s(Distance) +Congener +Sea Varident corCAR1
AIC for [PCB] in sediment	2964.3	2705.9	2695.9	2632.1
AIC for [PCB] in mussels	4361.4	4332.9	4320.6	2986.1

Table 3.2 *Four different model configurations, one in each column, were tested to infer spatiotemporal trends of the chlorophyll a concentrations ([CHFLa]) and sediment organic carbon content ([OC]) in the North Sea and Celtic Sea. Models were assessed using the Akaike information criterion (AIC). NC = algorithm did not converge.*

Model configuration	[CHFLa or OC] ~ s(Year|Sea) +s(Month) +Sea	[CHFLa or OC] ~ s(Year|Sea) +s(Month) +s(Distance) +Sea	[CHFLa or OC] ~ s(Year|Sea) +s(Month) +Sea Varident	[CHFLa or OC] ~ s(Year|Sea) +s(Month) +Sea Varident corCAR1
AIC for [CHFLa]	165.1	NC	136.0	129.3
AIC for [OC]	68.3	NC	81.7	83.7

3.3 Results and Discussion

3.3.1 Model selection

Spatiotemporal trends of polychlorinated biphenyl (PCB) concentrations in sediment (AIC = 2632; R^2 = 0.52), PCB concentrations in mussels (AIC = 2986; R^2 = 0.49; Table 3.1 and 3.3) and trends of chlorophyll a concentrations (AIC = 129; R^2 = 0.57; Table 3.2 and 3.3) were best modeled using model configuration 4. The organic carbon content in the sediment was best inferred using a less complex model, i.e. model configuration 1 (AIC = 68; R^2 = 0.30; Table 3.2 and 3.3). Although the spatiotemporal scale was large, these model configurations separated the interannual from the seasonal variability of the corresponding response variable (Table 3.3). The best model configuration for each variable was used for further analysis (Table 3.3). Note that seasonal temperature changes were implicitly accounted for by allowing seasonal fluctuations (by means of a smoother for month) in the model configurations.

Table 3.3 *P-values of the covariates used to infer spatiotemporal time trends of the polychlorinated biphenyl concentrations in sediment ($[PCB]_{sediment}$; n = 2,185) and mussels ($[PCB]_{mussel}$; n = 4,116), chlorophyll a concentration ($[CHFLa]_{water}$; n = 703) and the organic carbon content in the sediment ($[OC]_{sediment}$; n = 224) in the North Sea and Celtic Sea. Effects were tested at the 5% significance level. Model fit of the best model configuration for each variable (Table 3.1 and 3.2) is reported as the adjusted r-squared (R^2) and the Akaike information criterion (AIC).*

Model configuration	$[PCB]_{sediment}$ ~ s(Year|Sea) +s(Month) +s(Distance) +Congener +Sea	$[PCB]_{mussel}$ ~ s(Year|Sea) +s(Month) +s(Distance) +Congener +Sea	$[CHFLa]_{water}$~ s(Year|Sea) +s(Month) +Sea	$[OC]_{sediment}$ ~ s(Year|Sea) +s(Month) +Sea
Covariates				
Year: Celtic Sea	5.4×10^{-4}	$< 2.0 \times 10^{-16}$	7.4×10^{-8}	3.3×10^{-1}
Year: North Sea	$< 2.0 \times 10^{-16}$	$< 2.0 \times 10^{-16}$	$< 2.0 \times 10^{-16}$	2.3×10^{-7}
Month	$< 2.0 \times 10^{-16}$	$< 2.0 \times 10^{-16}$	$< 2.0 \times 10^{-16}$	7.2×10^{-5}
Distance to coast	$< 2.0 \times 10^{-16}$	4.1×10^{-2}		
Congener	8.7×10^{-6}	$< 2.0 \times 10^{-16}$		
Model fit				
R^2	0.516	0.493	0.566	0.302
AIC	2632	2986	129	68

3.3.2 Chlorophyll a and organic carbon time trends

Phytoplankton biomass has increased in the North Sea and Celtic Sea within the investigated time frame (Fig. 3.1). Between 1975 and 2012, interannual chlorophyll a concentrations doubled in the North Sea ($p < 2.0 * 10^{-16}$) and in the Celtic Sea ($p = 7.4 * 10^{-8;}$ Fig. 3.1). These results corroborate findings of other regional-scale studies (McQuatters-Gollop et al., 2011;

Wiltshire et al., 2008), while global-scale studies indicate a dissimilar trend (Boyce et al., 2010). The seasonal trend ($p < 2.0 * 10^{-16}$; Table 3.3) reflects the spring and fall bloom (Fig. 3.1 and 3.2) that is typical for temperate regions (Colebrook, 1979). In this context, macroscale research indicated large biogeographical plankton community shifts during the last 40 years as a consequence of climate change and species introductions (Alvarez-Fernandez and Riegman, 2012). However, the contribution of diatoms and dinoflagellates to the phytoplankton biomass maximally changed 4% in this period (Leterme et al., 2006; Rousseaux and Gregg, 2014). The moment of diatom peaks in spring and autumn remained stable between 1958 and 2002 (Edwards and Richardson, 2004) due to a large variation in diatoms' phenology. Also Wiltshire et al. (2008) reported that even under variable environmental conditions, the timing of phytoplankton blooms is fairly constant. The small confidence intervals around the seasonal trend of the chlorophyll a concentrations reflect the constant timing of phytoplankton blooms (Fig. 3.1).

The organic carbon content in North Sea sediment increased from 2.29 ± 1.69 g.100 g dry weight^{-1} in 1986 (n = 3) to 4.16 ± 1.79 g.100 g dry weight^{-1} in 2012 (n = 12), but remained relatively constant in the Celtic Sea within the investigated time frame (Fig. 3.3). The sediment organic carbon content showed seasonal variations in both seas ($p = 7.2 * 10^{-5}$; Fig. 3.3 and 3.4; Table 3.3), consistent with findings reported for the Southern Ocean (Nedwell et al., 1993) and the Atlantic Ocean (Helmke et al., 2005; Fischer et al., 2009).

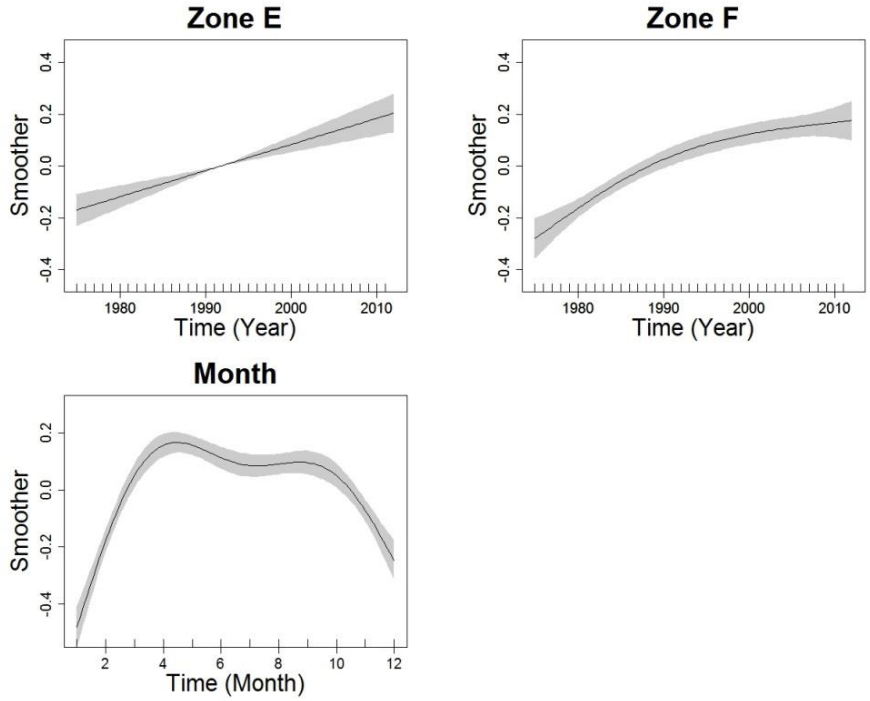


Figure 3.1 *Time smoothers revealing spatiotemporal trend of the chlorophyll a concentration in the Celtic Sea (E) and North Sea (F). The solid line is the predicted value of the response variable as a function of the x-axis and grey polygons indicate the pointwise 95% confidence intervals. The y-axis is a logarithmic smoother illustrating the relative effect of the x-axis on the chlorophyll a concentration. All trends are significant. The x-axis tick marks indicate measurements. Model configuration 4 was used to infer the spatiotemporal trend of the chlorophyll a concentration (Table 3.3).*

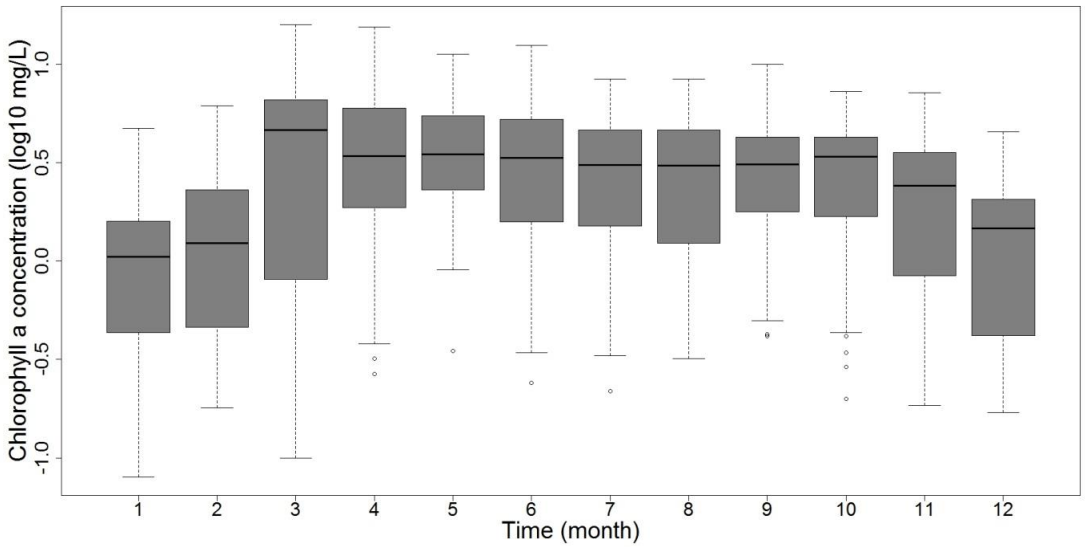


Figure 3.2 *Chlorophyll a concentrations (n = 703) plotted against the sampling month using box-and-whisker plots.*

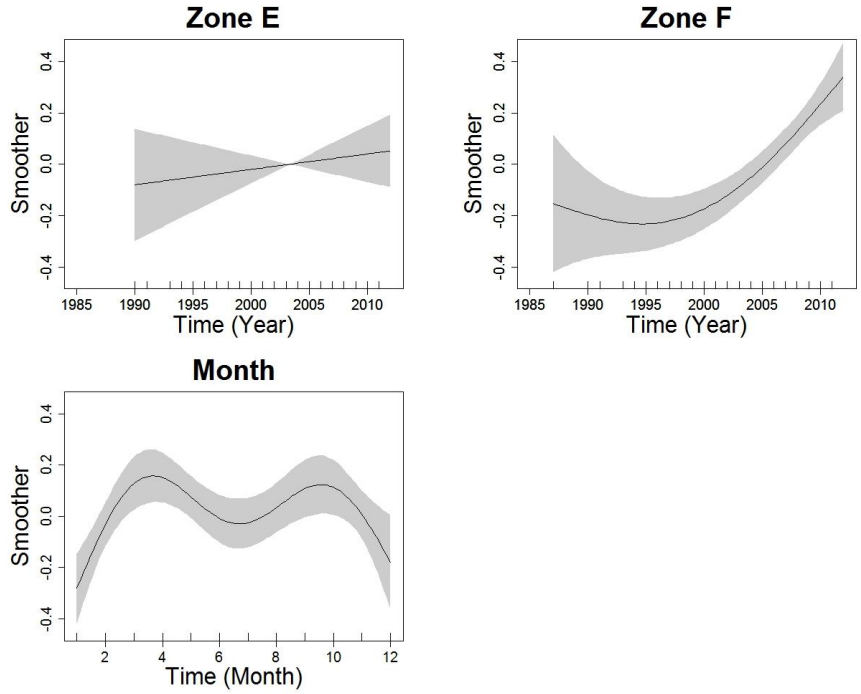


Figure 3.3 *Time smoothers revealing spatiotemporal trend of the sediment organic carbon concentrations in Celtic Sea (E) and North Sea (F). The solid line is the predicted value of the response variable as a function of the x-axis and grey polygons indicate the pointwise 95% confidence intervals. The y-axis is a logarithmic smoother illustrating the relative effect of the x-axis on the sediment organic carbon concentration. All trends are significant except for the one for the Celtic Sea. The x-axis tick marks indicate measurements. Model configuration 1 was used to infer the spatiotemporal trend of the sediment organic carbon concentrations (Table 3.3).*

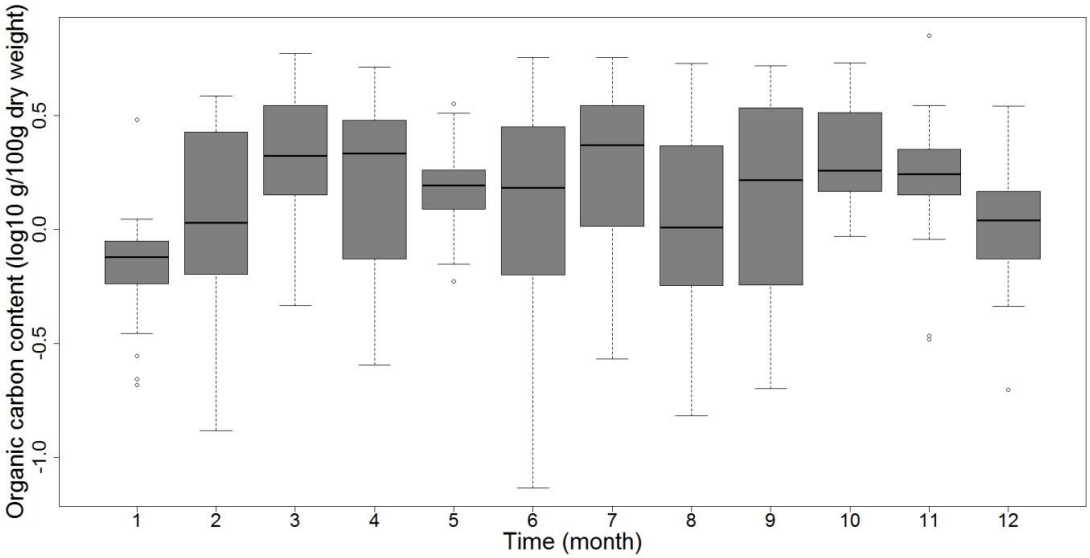


Figure 3.4 *Sediment organic carbon contents (n = 224) plotted against sampling month using box-and-whisker plots.*

3.3.3 Interannual polychlorinated biphenyl time trends

PCB concentrations in sediments show, less than PCB levels in mussels, decreasing interannual trends (Fig. 3.5 and 3.6) in response to reduced inputs (Breivik et al., 2007). In the North Sea, we did detect a four-fold decrease of the long-term (1990-2012) PCB concentrations in the sediment ($p < 2.0 * 10^{-16}$; Fig. 3.5; Table 3.3) consistent with the often reported decreasing polychlorinated biphenyl (PCB) time trends (Everaert et al., 2014; Gomez-Gutierrez et al., 2007; Hong et al., 2010). By contrast, PCB concentrations in the sediment of the Celtic Sea were stable between 1991 and 2012 ($p = 5.4 * 10^{-4}$; Fig. 3.5; Table 3.3). The main reason is that most of the analyzed data in the Celtic Sea were close to industrialized estuaries and in harbours (Fig. B.3). In the proximity of harbours often elevated (compared to open water conditions) PCB concentrations are found due to historical pollution of industrial activities (Gomez-Gutierrez et al., 2007). For example, in the Western Scheldt estuary, Everaert et al. (2014) found relatively stable trends of PCB concentrations in the sediment between 1991 and 2010 (cfr. chapter II).

PCB concentrations in soft tissue of mussels exhibited a decreasing time trend in the North Sea and in the Celtic Sea ($p < 2.0 * 10^{-16}$; Fig. 3.6; Table 3.3), confirming other long-term observations in marine biota (Roose et al., 1998).

Although we obtained a good model fit, residual diagnostics (Fig. B.5 and B.6) show that low PCB concentrations were predicted too high. We plotted the model residuals against the corresponding congeners (Desforges et al., 2014), but the unexplained variability was not related to congener-specific elements (Fig. B.7 and B.8). When plotting the model residuals versus the year in which the samples were taken, we observed a long-term fluctuating trend with a periodicity of ca. eight years (Fig. B.9 and B.10). A potential explanation for these fluctuating model residuals is the influence of large-scale climatic oscillations on the PCB concentrations (Fig. B.11 and B.12). Recently, Everaert et al. (2014) found an interannual oscillation of the PCB concentration in the sediments along the Belgian coast similar to the period of the North Atlantic Oscillation (NAO). Also at larger spatial scales, interannual fluctuations of POP concentrations were linked to climatic oscillations such as the el Niño southern oscillation (ENSO) and the NAO. For example, in the Great Lakes region Ma et al. (2004) found increased POP levels in air under el Niño events and attributed this to revolatilization from contaminated soil due to air temperature increases. Zhai et al. (2013) reported that the ratio of diatoms to phytoplankton biomass is related with the NAO. In years with high NAO values, relatively more diatoms are present, stimulating the effectiveness of the biological pump and resulting in increased PCB concentrations in the sediment. As such, at least part of the unexplained variability in the PCB concentrations might be related to these large-scale climatic oscillations (Fig. B.9 - B.12).

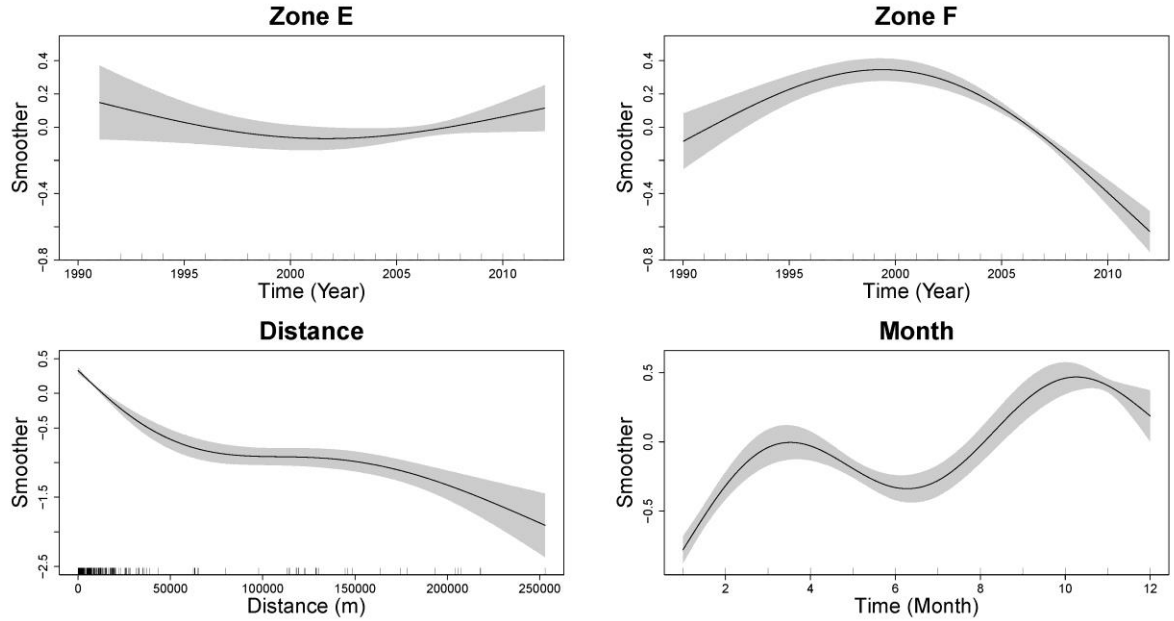


Figure 3.5 *Time smoothers revealing spatiotemporal trend of the PCB concentrations in the sediment of the Celtic Sea (E) and North Sea (F). The solid line is the predicted value of the response variable as a function of the x-axis and grey polygons indicate the pointwise 95% confidence intervals. The y-axis is a logarithmic smoother illustrating the relative effect of the x-axis on the PCB concentration in the sediment. All trends are significant. The x-axis tick marks indicate measurements. Model configuration 4 was used to infer the spatiotemporal trend of the PCB concentrations in the sediment.*

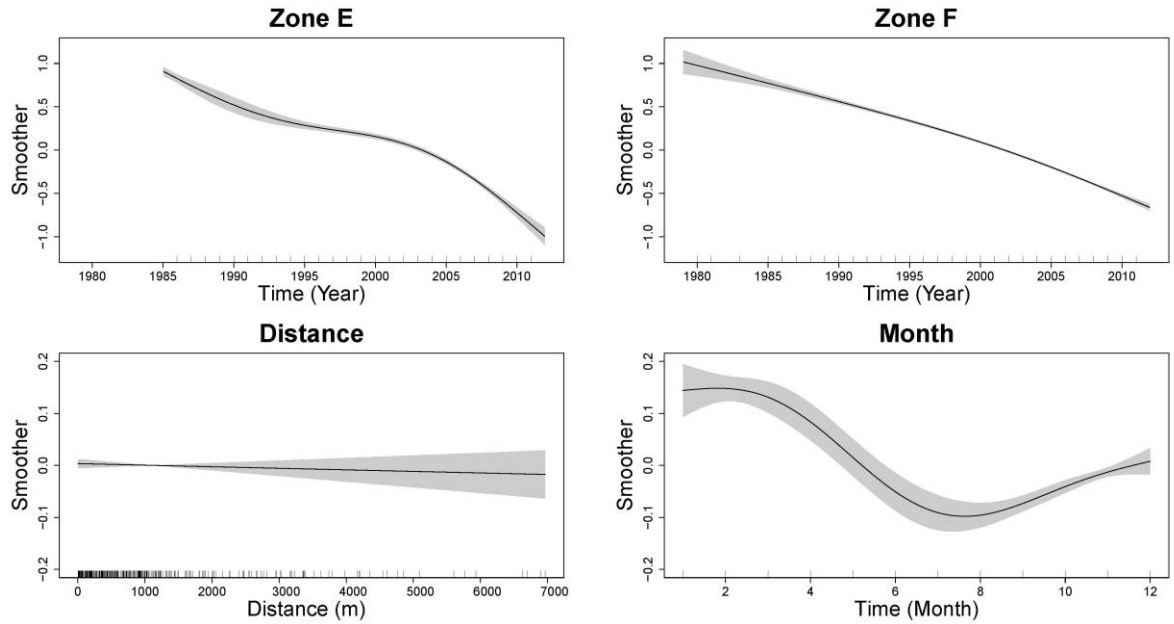


Figure 3.6 *Time smoothers revealing spatiotemporal trend of the PCB concentrations in mussels of the Celtic Sea (E) and North Sea (F)). The solid line is the predicted value of the response variable as a function of the x-axis and grey polygons indicate the pointwise 95% confidence intervals. The y-axis is a logarithmic smoother illustrating the relative effect of the x-axis on the PCB concentration in the mussels. All trends are significant. The x-axis tick marks indicate measurements.*

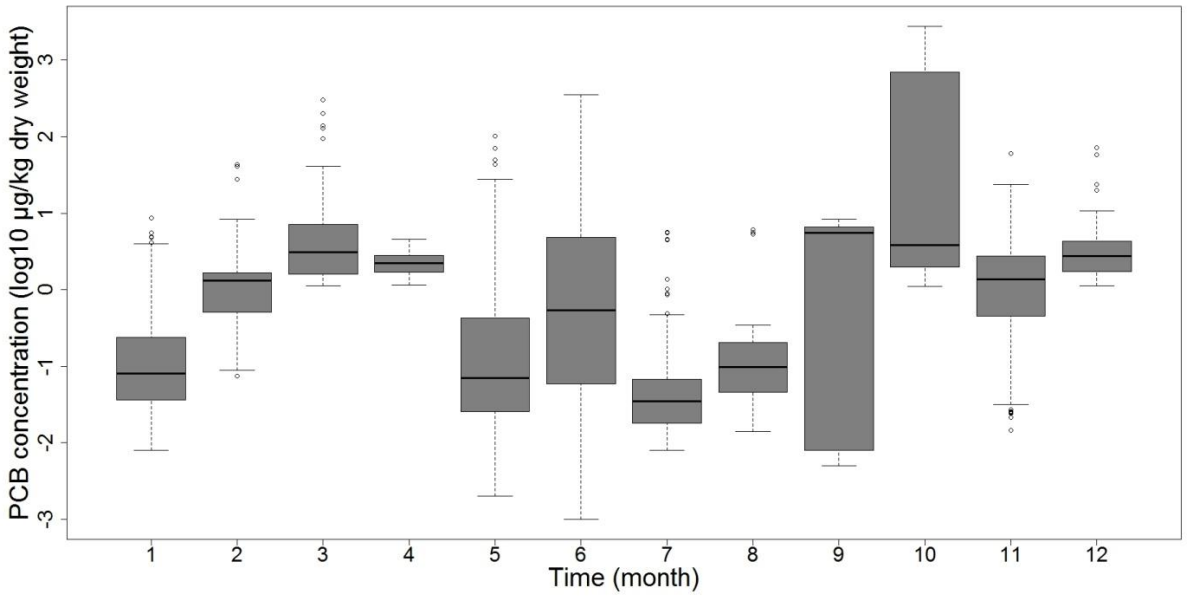


Figure 3.7 *PCB concentration in sediment (n = 2,185) plotted against sampling month using box-and-whisker plots.*

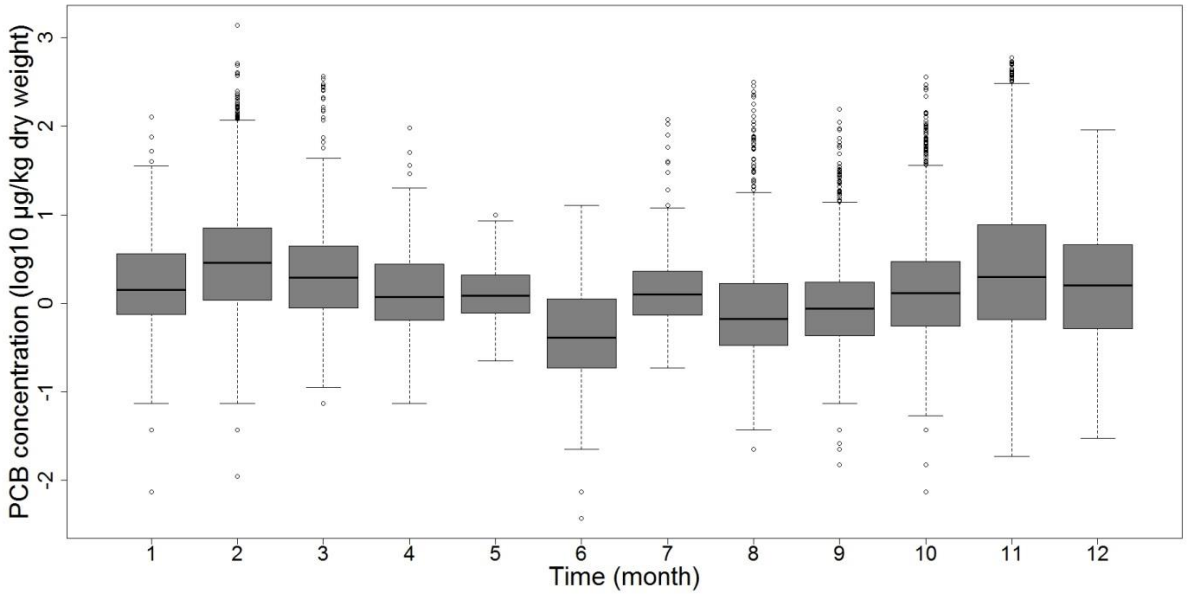


Figure 3.8 *PCB concentration in mussels (n = 4,116) plotted against sampling month using box-and-whisker plots.*

3.3.4 Seasonal polychlorinated biphenyl time trends

PCB concentrations in the sediment exhibited a distinct seasonal pattern, with peaks in March-April and September-October ($p < 2.0 * 10^{-16}$; Fig. 3.5 and 3.7), and decreased from the coast towards more offshore areas ($p < 2.0 * 10^{-16}$; Fig. 3.5; Table 3.3). The results are consistent with observations in the southern part of the North Sea (Laane et al., 1999) and in the Scottish marine environment (Webster et al., 2011).

PCB concentrations in mussels varied seasonally with relatively low concentrations observed in summer and highest concentrations in winter months ($p < 2.0 * 10^{-16}$; Fig. 3.6 and 3.8; Table 3.3). Since all mussels were sampled close to the coast (Fig. B.4), we did not observe a spatial gradient in the PCB concentrations in mussels ($p = 4.1 * 10^{-2}$; Fig. 3.6).

3.3.5 Coupling between phytoplankton and polychlorinated biphenyl seasonal dynamics

We found strong and significant correlations between seasonal changes of phytoplankton biomass (i.e. chlorophyll a concentrations) and seasonal changes of PCB concentrations in the sediment compartments of the two seas (Table 3.4). In the Celtic Sea, chlorophyll a trends and sediment organic carbon trends correlated positively with the PCB concentrations in the sediment (r = 0.61 and r = 0.74, respectively) and correlated negatively with the PCB concentrations in mussels (r = -0.33 and r = -0.52, respectively) (Table 3.4). Correlations in the North Sea were comparable to those derived for the Celtic Sea (Table 3.4). Furthermore, chlorophyll a correlated positively with the organic carbon content of the sediment (r = 0.71) and PCB concentrations in mussels were negatively correlated with PCB concentrations in sediment (r = -0.78 and r = -0.76 in the North and Celtic Sea, respectively).

Table 3.4 *Correlation coefficients between the polychlorinated biphenyl concentrations in sediment ($[PCB]_{sediment}$) and mussels ($[PCB]_{mussel}$), chlorophyll a concentration ($[CHFLa]_{water}$) and the organic carbon content in the sediment ($[OC]_{sediment}$) in the North Sea and Celtic Sea. Corresponding p-values are given between brackets and effects were tested at the 5% significance level. Significant relations are indicated in bold.*

	$[PCB]_{sediment}$	$[PCB]_{mussel}$	$[CHFLa]_{water}$
North Sea			
$[PCB]_{sediment}$	-		
$[PCB]_{mussel}$	**-0.78** (0.003)	-	
$[CHFLa]_{water}$	**0.48** (0.011)	-0.34 (0.270)	-
$[OC]_{sediment}$	**0.77** (0.003)	-0.50 (0.095)	**0.71** (0.010)
Celtic Sea			
$[PCB]_{sediment}$	-		
$[PCB]_{mussel}$	**-0.76** (0.004)	-	
$[CHFLa]_{water}$	**0.61** (0.037)	-0.33 (0.290)	-
$[OC]_{sediment}$	**0.74** (0.006)	-0.52 (0.085)	**0.71** (0.010)

The phytoplankton blooming events in spring and autumn (Fig. 3.1 and 3.2) correspond to the annual maxima of sediment organic carbon contents (Fig. 3.3 and 3.4) and maxima of PCB concentrations in the sediment (Fig. 3.5 and 3.7). Thus, the seasonal PCB dynamics are intimately linked to seasonal phytoplankton dynamics, consistent with the findings of Konat and Kowalewska (2001) and Galban-Malagan et al. (2012).

The correlation between the phytoplankton blooming events and the annual maxima of sediment organic carbon suggests that near-surface changes propagate rapidly into deeper water layers (Altabet et al., 1991). This is most likely due to diatom-dominated spring and autumn blooms (McGlade, 2002) with sinking rates of diatoms up to several hundreds of meters per day (Smetacek et al. (1985) and the shallow average depth of the North Sea (95 m) and Celtic Sea (120 m). Between May and July we observed relatively low PCB

concentrations in the sediment (Fig. 3.5). The latter was not related to the degradation of the PCBs as these substances are highly persistent with sediment half-lifes of several years (EPI Suite™; http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm). Most likely, between May and July the mineralization of organic matter in the sediment is faster than the sedimentation of new organic matter under the form of slow sinking (dino)flagellates (Smetacek, 1985; Middelburg et al., 1996). As such, during summer, the organic carbon content in the sediment – and its associated PCB absorbing capacity – is reduced (Fig. 3.3). To further demonstrate the cycling of PCBs between different environmental compartments, we analyzed the dissolved concentrations of PCB28 in the North Sea and Celtic Sea. Although PCB measurements in the water samples are scarce (n = 151; Table B.4), we found that PCB concentrations in water were lower in summer months than in winter months (Fig. 3.9). Following the biological pump theory, PCBs are taken up by phytoplankton and settle down in the sediment. As such, when phytoplankton blooms (Fig. 3.1), PCB concentrations in the sediment increase (Fig. 3.5) and freely dissolved concentrations of PCBs are depleted (Fig. 3.9).

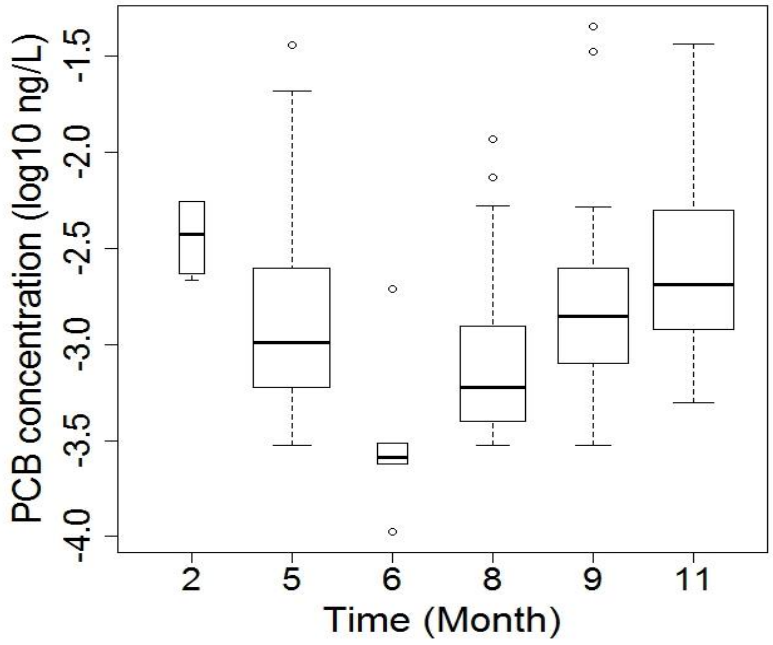


Figure 3.9 *Monthly fluctuation of the concentrations polychlorinated biphenyl congener 28 (PCB28) in sea water samples (n = 151 between 2005 and 2011) from the Celtic Sea and North Sea. Although sea water samples were not available for each month, it is shown that dissolved concentrations of PCB28 in winter are higher than summer concentrations. The width of the boxplots is proportional to the amount of samples for each month (i.e. February: n = 4, May: n = 38, June: n = 6, August: n = 28, September: n = 33 and November: n = 42).*

Explaining the biogeochemical processes underlying the seasonal variability of PCB concentrations in mussels (Fig. 3.6 and 3.8) is more complex. Schwarzenbach et al. (2005) concluded that bivalves acquire organic chemicals mainly from their diet, i.e. PCB-enriched phytoplankton and sediment result in increased internal PCB concentrations in mussels. According to this hypothesis, the negative correlation between the PCB concentrations in the sediment and the PCB concentrations in the mussels is contra-intuitive (Table 3.4). A second theory is that the freely dissolved phase is the most important exposure route for mussels to

organic chemicals (Connolly and Pedersen, 1988), especially when suspended solids are low (Bruner et al., 1994). We found that exposure to relatively clean water (second theory; Connolly and Pedersen, 1988; Bruner et al., 1994) affected the PCB concentrations in mussels more than the uptake of PCB-enriched phytoplankton (first theory). The negative correlation between the PCB concentrations in mussels and the PCB concentrations in sediment (Table 3.4) is reflected in the unprocessed data (Fig. 3.8 and 3.9). We observed about one order of magnitude difference between summer and winter freely dissolved concentrations of PCB28 (Fig. 3.9), but PCB concentrations in mussels only changed with a factor of about five (Fig. 3.8). This might indicate that also other processes (e.g. filtration of PCB-enriched phytoplankton in summer) or other environmental factors influence the seasonality of PCB concentrations in mussels. In this respect, increased hibernal internal POP concentrations in biota were observed in the Arctic marine and terrestrial environment and were attributed to food shortage and a faster metabolization of the accumulated fatty tissues in winter (De Laender et al., 2010; Bustnes et al., 2011). Also the loss of fatty tissue due to spawning activity in summer plays an important role in the internal POP concentrations of mussels (Gabbott, 1983). Overall, in spring, mussels filter PCB-enriched phytoplankton, which will increase their internal PCB content (Schwarzenbach et al., 2005). However, in summer when freely dissolved concentrations are low, the accumulated PCBs will partition back to the water phase (Connolly and Pedersen, 1988).

3.4 Conclusion

We present the first large-scale spatiotemporal coupling between seasonal dynamics of phytoplankton, sediment organic carbon and PCB concentrations. Seasonal changes of POP concentrations due to the biological pump have been theoretically described (Gasol et al., 1997) and observed on relatively small spatiotemporal scales (Galban-Malagan et al., 2012; Berrojalbiz et al., 2011). However, our study is the first to separate long-term interannual changes and spatial changes from seasonal fluctuations so that the dynamic behaviour of PCB concentrations, chlorophyll a and organic carbon can be compared. Despite other factors affecting POP cycling (e.g. physical-chemical conditions, wind, currents, temperature fluctuations; Dachs et al., 2002; Lohmann and Belkin, 2014), we show that the sequestration of PCBs is seasonal and our results strongly suggest the influence of phytoplankton dynamics on this seasonality.

4

Comparison of long-term series of polychlorinated biphenyls concentrations of the North Sea and Celtic Sea with environmental assessment criteria

Redrafted from:
Gert Everaert, Frederik De Laender, Peter L.M. Goethals, Colin R. Janssen. Potential risk of polychlorinated biphenyls in North Sea and Celtic Sea using multidecadal data. Marine Environmental Research. To be submitted.

Abstract

We assessed the ecological risk of seven marker polychlorinated biphenyls (PCBs) in North Sea and Celtic Sea sediment and mussel samples. To do so, we compared PCB concentrations with environmental assessment criteria (EAC) considering the spatiotemporal variability of PCB concentrations. Furthermore, we showed how the -one order of magnitude- wide range of EAC may influence the outcome of the environmental risk assessment (risk vs. no risk). We found that risk quotients (RQs) of PCBs in North Sea and Celtic Sea sediments' primarily depend on the location of the monitoring site, i.e. the closer to the coast, the higher the RQ. Especially in summer, when PCB concentrations in sediments are high, PCBs present in marine coastal sediments may pose an environmental risk. By contrast, RQs in mussel primarily depend on the interannual trends of PCB concentrations and do not depend on the distance of the sampling location to the coast. At present, in the Celtic Sea, RQs in mussels are below the value of 1, suggesting no potential environmental risk. In the North Sea, however, RQs calculated based on the most stringent EAC value still exceed a value of 1. Our results demonstrate that the spatiotemporal variability of PCB concentrations in different environmental compartments should be considered in future environmental risk assessments.

4.1 Introduction

Adverse biological effects of persistent organic pollutants on marine ecosystems are increasingly reported (e.g. Widdows et al., 2002; Langston et al., 2012; Mearns et al., 2013). Understanding the causes of these effects is crucial to take informed management actions (OSPAR, 2010). The presence of organic chemicals due to natural and human-induced chemical processes lists amongst the potential causes (Halpern et al., 2008), but the overall impact of organic chemicals on the marine environment is largely unknown (Rockström et al., 2009).

Concentrations of organic chemicals in the marine environment are location- and time-dependent (Wu et al., 2008). On a spatial scale, polychlorinated biphenyl (PCB) concentrations, which are often used as model compounds for organic chemicals (Schuster et al., 2010; De Laender et al., 2011; Everaert et al., 2014), decrease from inshore towards offshore sampling locations (Gomez-Gutierrez et al., 2007). For example, freely dissolved concentrations of seven PCB congeners in the open Atlantic Ocean (0.071-1.7 $pg.L^{-1}$, Gioia et al., 2008) are up to three orders of magnitude lower than the concentrations found in Scottish estuaries (0.07-0.13 $ng.L^{-1}$; Emelogu et al., 2013). Along the Belgian coast, concentrations of organic chemicals in harbor sediments are about 15-fold higher than concentrations at coastal monitoring sites (Everaert et al., 2014). As such, especially in coastal areas (Widdows et al., 2002; Bellas et al., 2014), in estuaries (Webster et al., 2011), in bays (Szlinder-Richert et al., 2012; Zhang et al., 2014) and in harbors (Stronkhorst and van Hattum, 2003; Montero et al., 2013) PCBs can pose risks to marine life by exerting toxicological effects. Superimposed to the spatial variability, concentrations of organic chemicals change on a multidecadal (Hung et al., 2005; Everaert et al., 2014), on an interannual (Roose et al., 1998; Gomez-Gutierrez et al., 2007) and on a seasonal scale (Galban-Malagon et al., 2012). Furthermore, Ma et al. (2010) reported multidecadal fluctuations in PCB concentrations due to climatic oscillations such as the North Atlantic Oscillation (NAO) and the El Nino-Southern Oscillation (ENSO). High temperatures associated with positive NAO and ENSO phases enhance the volatilization of PCBs from historic reservoirs (e.g. marine sediments; Ma et al., 2004). On a seasonal scale, PCB concentrations are tightly coupled to phytoplankton growth dynamics (Dachs et al., 1999; Nizzetto et al., 2012). Overall, concentrations of organic chemicals at which marine organisms are exposed are spatiotemporally variable.

The spatiotemporal variability of organic chemicals is a matter of great concern in monitoring and assessment of marine chemical pollution (Roose et al., 2011). In chemical monitoring campaigns, focus is typically on the long-term concentration changes to detect possible deterioration or improvement of the pollution status. Given the spatiotemporally changing concentrations of organic contaminants, it is crucial to be able to separate interannual changes from seasonal and spatial changes. However, to date the possible implications of the spatiotemporal variable concentrations of organic chemicals for ecological risk assessment is poorly studied. Therefore, the aim of the present research is to examine the implications of spatiotemporal variable PCB concentrations on environmental risk assessment. To cover the different sources of variability, large numbers of sampling data are needed at large spatiotemporal scales (Roose et al., 2011; Widdows et al., 2002; Webster et al., 2011; Bellas

et al., 2014). Therefore focus is on the greater North Sea (including the Kattegat) and the Celtic Seas (including the western part of Scotland) since these seas have a long history of chemical pollution and are globally amongst the most intensively monitored seas.

4.2 Materials and methods

We infer spatiotemporal trends of polychlorinated biphenyl (PCB) concentrations in sediment and biota (Fig. 3.5 and 3.6) and compare these trends with sediment and biota quality thresholds for the marine environment.

4.2.1 Data

Data were obtained from the International Council for the Exploration of the Sea (ICES, http://www.ices.dk/). Focus was on seven PCB congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180). Details on the data selection and the amount of data retained after each step can be found in Chapter 3. The data selection resulted in two sets of data: PCB concentrations in sediments (n = 2,185) and PCB concentration in mussels (n = 4,116).

4.2.2 Model

We applied an additive (mixed) model (A(M)M) to infer spatiotemporal trends of PCB concentrations in sediments and mussels. Covariates used to model the trends were: the time of sampling (as year and month), the sampling location (name of the sea), the congener name, a smoother for the distance of the sampling location to the coast, and if appropriate a variance structure to allow a flexible residual spread and a continuous correlation structure to consider autocorrelation between sequential observations. Multiple model configurations were tested. A detailed overview and discussion of the model configurations, model parameterizations and model validations can be found in Chapter 3.

4.2.3 Risk quotient

Spatiotemporal trends of PCBs in sediment were compared with environmental assessment criteria (EAC) proposed by the OSPAR commission (1.00 – 10.0 µg.kg dw^{-1}) and the actual threshold (2.60 µg.kg dw^{-1}) derived from Plesha et al. (1988) by means of assessment factors (OSPAR, 1998; see paragraph 4.3.3 for a critical analysis of the EAC). As such, risk quotients (RQs) of $\sum PCB_7$ in the North Sea and Celtic Sea sediment were calculated using 1.00 µg.kg dw^{-1} (lower confidence interval), 2.60 µg.kg dw^{-1} (actual threshold) and 10.0 µg.kg dw^{-1} (upper confidence interval). We calculated the RQ as:

$$RQ = \frac{\mathrm{PEC}}{\mathrm{EAC}} \qquad \text{(Eq. 4.1)}$$

with PEC the predicted environmental concentrations obtained from the spatiotemporal trends; and EAC the environmental assessment criteria of the OSPAR commission. For sediment concentrations, we assessed the effect of two distances on the RQs: at the coastline (0 km) and at open sea (200 km offshore). RQs were plotted over time and the lower confidence interval based RQs (1.00 µg.kg dw^{-1}) and upper confidence interval based RQs

(10.0 µg.kg dw^{-1}) were used as borders (Fig. 4.1). When the value of the RQ is larger or equal to 1, a potential environmental risk is identified (Ghekiere et al., 2013).
Concentrations of $\sum PCB_7$ in the soft body of the blue mussel (*Mytilus edulis*) were compared (Eq. 4.1) with the lower limit of the EAC (5.00 µg.kg dw^{-1}), the upper limit of the EAC (50.0 µg.kg dw^{-1}) and the actual threshold (22.8 µg.kg dw^{-1}) as derived from Leonards et al. (1994) (OSPAR, 1998). For PCB concentrations in mussels no spatial gradient was detected (Fig. 3.6), hence we only calculated RQs for sampling locations along the coast (0 km). RQs were plotted over time and the lower confidence interval based RQs (5.00 µg.kg dw^{-1}) and upper confidence interval based RQs (50.0 µg.kg dw^{-1}) were used as borders (Fig. 4.2). In a final step, we compared PCB concentrations in the sediment to consensus-based sediment effects ($\sum PCB_{total}$ = 48.0 ± 47.0 µg. kg dw^{-1}, n =5) for marine and estuarine ecosystems (MacDonald et al., 2000).

4.3 Results and Discussion

4.3.1 Spatiotemporal trends of polychlorinated biphenyls in marine sediments

Concentrations of polychlorinated biphenyls (PCBs) in sediments of the North Sea and Celtic Sea are spatiotemporally variable. We observed a decreasing pollution gradient from coastal towards offshore monitoring sites: the closer to the coast, the higher the PCB concentrations ($p < 2.0 * 10^{-16}$; Fig. 3.5). At present, PCB concentrations at open sea (0.46 ± 0.21 µg.kg dw^{-1} and 0.05 ± 0.02 µg.kg dw^{-1} for the Celtic Sea and the North Sea, respectively) are 33-fold lower than those at sampling locations along the coast (15.1 ± 6.3 µg.kg dw^{-1} and 1.66 ± 0.69 µg.kg dw^{-1} for the Celtic Sea and North Sea, respectively). PCB concentrations in marine sediment showed interannual variability in North Sea with four-fold decreasing concentrations between 1990 and 2012 ($p < 2.0 * 10^{-16}$; Fig. 3.5). However, in the Celtic Sea sediments' PCB concentrations were relatively stable between 1991 and 2012 ($p = 5.4 * 10^{-4}$). Furthermore, PCB concentrations in the sediment exhibited a distinct seasonal pattern, with peaks in March-April and September-October ($p < 2.0 * 10^{-16}$; Fig. 3.5).

4.3.2 Spatiotemporal trends of polychlorinated biphenyls in marine mussels

PCB concentrations in mussels show, more than PCB levels in sediments, decreasing interannual trends ($p < 2.0 * 10^{-16}$; Fig. 3.6). Also on a seasonal level, PCB concentrations in mussels were fluctuating, i.e. within one sampling year lowest concentrations were observed in summer ($p < 2.0 * 10^{-16}$). At spatial scale no trend was observed ($p = 4.1 * 10^{-2}$), i.e. PCB concentrations in mussels collected at the coastline are similar those collected more offshore.

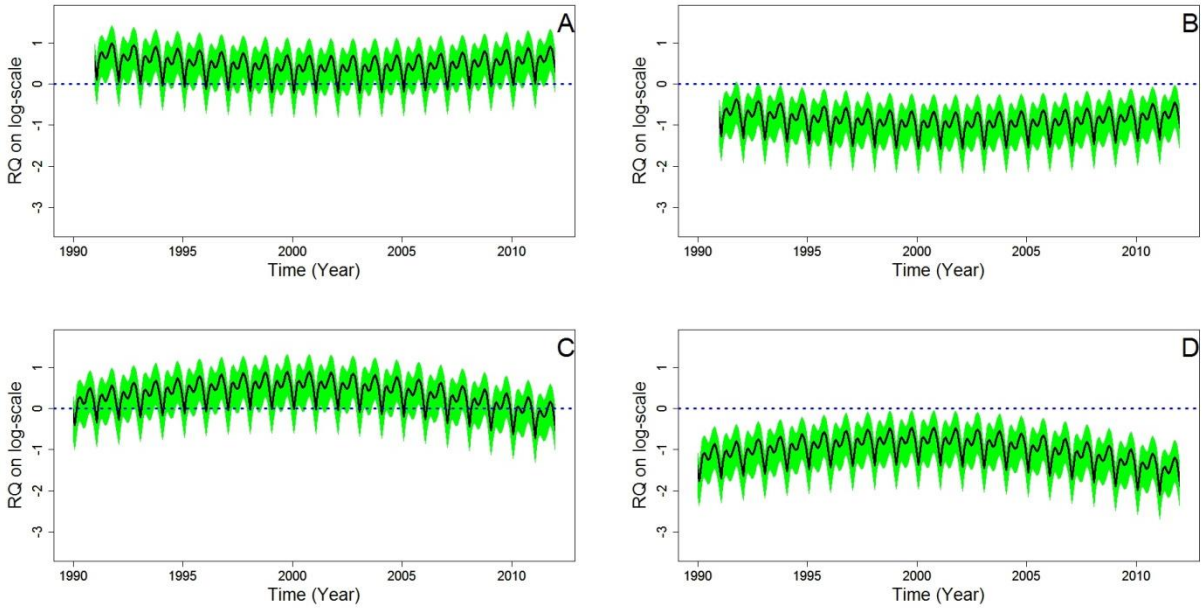


Figure 4.1 *Spatiotemporal trends of sediment PCB concentrations in the Celtic Sea (panel A and B) and the North Sea (panel C and D) in function of the distance of the sampling locations to the coast: 0km (panel A and C) and 200 km (panel B and D). Polychlorinated biphenyls (PCBs) data included concentrations of seven congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) which were compared with the OSPAR sediment quality thresholds (1.00 – 10.0 µg.kg dw^{-1}; green polygon) and the actual quality threshold (2.60 µg.kg dw^{-1}; solid line) inferred from Plesha et al. (1988). The horizontal dashed line represents a risk quotient (RQ) value of 1.*

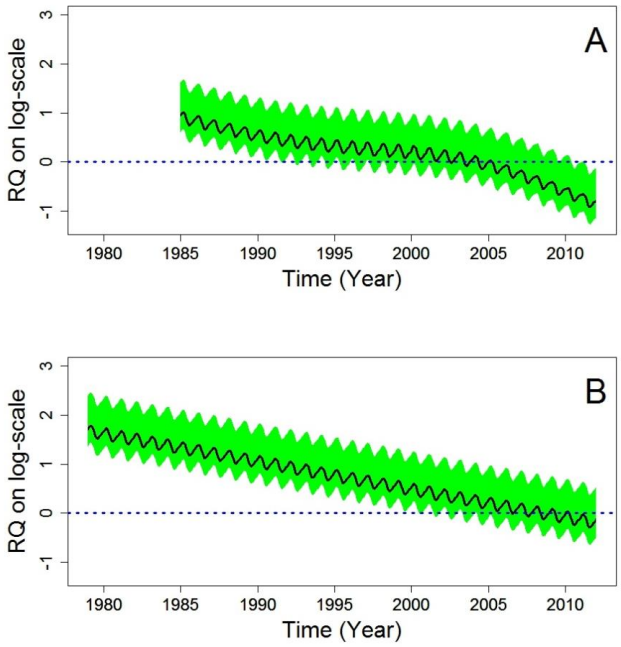


Figure 4.2 *Spatiotemporal trends of mussel PCB concentrations in the Celtic Sea (panel A) and the North Sea (panel B). Polychlorinated biphenyls (PCBs) data included concentrations of seven congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) which were compared with the OSPAR sediment quality thresholds (5.00 – 50.0 µg.kg dw^{-1}; green polygon) and the actual quality threshold (22.8 µg.kg dw^{-1}; solid line) inferred from Leonards et al. (1994). The horizontal dashed line represents a risk quotient (RQ) value of 1.*

4.3.3 Risk quotients

Organic chemicals are a potential cause of adverse ecological effects in the marine environment (Fig. 4.1). By comparing PCB concentrations measured in marine sediment with the corresponding quality threshold concentrations, we found that coastal sediments in the North Sea and Celtic Sea may give rise to ecotoxicological effects (Fig. 4.1). The risk quotients (RQs) of PCBs in North Sea and Celtic Sea sediments' primarily depend on the distance of the monitoring sites to the coastline (Fig. 4.1). RQs of PCB concentrations in sediments at open sea (200km offshore) are below 1 (Fig. 4.1B and 4.1D). However, in sediments monitored close to the coastline it is likely that RQs exceed the value of 1 (Fig. 4.1A and 4.1C). This is consistent with findings of Szlinder-Richert et al. (2012) in sediment of the Baltic Sea and Stronkhorst and Van Hattum (2003) in Dutch coastal harbor sediments. Secondly, RQs depend on the interannual fluctuations of PCB concentrations. We observe that RQs of North Sea sediment are decreasing from the year 2000 onwards (Fig. 4.1). Furthermore, within one phytoplankton growth season, RQs change considerably and are, in case of stable interannual trends, the second most important driver of RQs (Fig. 4.1).

RQs of the soft-body of the blue mussel primarily depend on the interannual trends of PCB concentrations (Fig 4.2). The effect of seasonality on the RQs is relatively small in comparison to the interannual changes. RQs in mussels do not depend on the distance of the sampling location to the coast. At present, in the Celtic Sea, RQs are below the value of 1, and thus not associated with potential environmental risks (Fig. 4.2A). In the North Sea, however, RQs calculated based on the most stringent criteria (5.00 µg.kg dw^{-1}) still exceed a value of 1, indicating a potential risk (Fig. 4.2B). Overall, RQs in mussels were clearly decreasing at an interannual scale (Fig. 4.2) and their current ecotoxicological risk is low in both regional seas (Fig. 4.2). In this context, Webster et al. (2009) reported that PCB concentrations in mussels were below quality thresholds along Scottish coast and Leon et al. (2013) did not observe environmental risk of PCB concentrations in bivalves in the Mediterranean Sea. Apart from the interannual changes, we showed that also seasonally variable PCB concentrations affect the RQs. In marine sediments we found that RQs peaked in spring and autumn following the phytoplankton blooms (Fig. 4.1). Seasonal effects were less important for RQs in mussel samples than for sediment samples (Fig. 4.2). A study of Webster et al. (2009) reports seasonally variable concentrations of polycyclic aromatic hydrocarbon (PAHs). Highest PAH concentrations were reported between November and March, but the reported concentrations remained throughout the entire year far below the quality thresholds (Webster et al., 2009).

Confidence intervals around RQs are large, indicating the large degree of uncertainty associated with the environmental assessment criteria (EAC) and the suggested adverse effect (Fig. 4.1 and 4.2). EAC define a -one order of magnitude wide- range of concentrations below which pollution levels are regarded as not harmful for the marine ecosystem. However, the consequences of PCB concentrations that exceed quality thresholds are unclear (Brown et al., 2013). For example, in Canada average sediment PCB concentrations exceeded the sediment quality thresholds by 41-fold, but sediment toxicity testing and a benthic community survey showed no evidence of adverse effects (Brown et al., 2013). According to Langston et al.

(2012) quality thresholds are only indicators to potential ecotoxicological impact as they are derivatives from ecotoxicological data (OSPAR, 1998).
EAC for the sum of concentrations of seven PCB ($\sum PCB_7$) in marine sediments were based on a spiked sediment test with organic contaminants of Plesha et al. (1988). In the experimental approach, Plesha et al. (1988) concluded an acute toxicity effect on the amphipod *Rhepoxynius abronius* at $\sum PCB_{total}$ of 5.20 mg.kg dw^{-1}. Based on the assumption that $\sum PCB_7$ is 50% of the $\sum PCB_{total}$ and after applying an assessment factor of 1000 (OSPAR, 1988) a threshold value of 2.60 µg.kg dw^{-1} was obtained. Considering a range of an order of magnitude around this value, the EAC for $\sum PCB_7$ in marine sediments were 1.00 and 10.0 µg.kg dw^{-1} (OSPAR, 1998). Similarly, the EAC of the sum of concentrations of seven PCB ($\sum PCB_7$) in mussels were based on a review of Leonards et al. (1994) about adverse effects of PCBs on mink (*Mustela vison)*. Leonards et al. (1994) reported an effect concentration (EC1) of 145 µg.kg ww^{-1} for mink. This threshold concentration was recalculated, based on a division by a factor 2 to convert total PCB concentrations to $\sum PCB_7$, an assessment factor of 10 and extra extrapolation factor to obtain concentrations in prey organisms, to obtain threshold concentrations of 7.30 µg.kg ww^{-1} for fish and 22.8 µg.kg dw^{-1} for mussel. The latter values finally resulted in EAC for fish of 1.00-10.0 µg.kg ww^{-1} and for mussel of 5.00-50.0 µg.kg dw^{-1} (OSPAR, 1998). Overall, EAC are associated with a large degree of uncertainty (Wu et al., 2008) as they are derived using assessment factors to experimental ecotoxicity values. Therefore, EAC that are currently used in environmental management need an urgent update as they are: (1) more than 20 years old; (2) based on simple toxicity tests; (3) include a large amount of assumptions; and (4) are, in the case of EAC for mussels and fish, based toxicity tests with terrestrial organisms which are not representative for the marine environment. Based on the large degree of uncertainty associated with the quality thresholds there is a need for more theoretical and experiment-based data to reduce the use of assessment factors. Additional ecotoxicological data are needed to allow a better linkage between RQs and -potential- adverse effects. Therefore, RQs above 1 cannot be considered as evidence of adverse biological effects (Wu et al., 2008), but should trigger concern and initiate biological investigations (Langston et al., 2012).

MacDonald et al. (2000) discussed the multitude of quality thresholds that have been developed to assess marine and estuarine sediments. To this end, by integrating multiple quality thresholds MacDonald et al. (2000) proposed a consensus-based sediment effect concentration for $\sum PCB_{total}$ of 48.0 ± 47.0 µg.kg dw^{-1} for $\sum PCB_{total}$. Assuming that $\sum PCB_7$ is 50% of the $\sum PCB_{total}$ (OSPAR, 1998) we obtain a threshold of 24.0 ± 23.5 µg.kg dw^{-1} for $\sum PCB_7$. Hence, according to MacDonald et al. (2000), 0.50 µg.kg dw^{-1} (24.0 – 23.5; lower confidence interval), 24.0 µg.kg dw^{-1} (actual threshold) and 47.5 µg.kg dw^{-1} (24.0 + 23.5; upper confidence interval) delimit the consensus-based sediment effect concentrations for marine and estuarine ecosystems for $\sum PCB_7$. Note that the EAC (1.00 – 10.0 µg.kg dw^{-1}) is at the lower end of the range proposed by MacDonald et al. (2000). When applying this range (24.0 ± 23.5 µg.kg dw^{-1}) to our data using equation 4.1, we observe that it is more likely that RQs are below the value of 1 (Fig. 4.3).

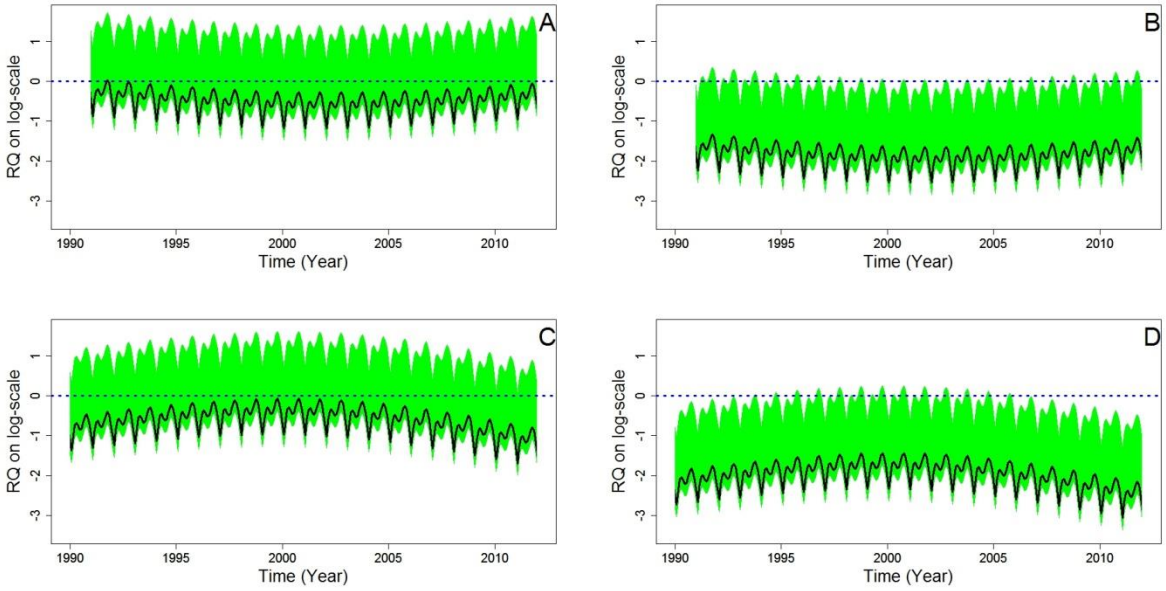


Figure 4.3 *Spatiotemporal trends of sediment PCB concentrations in the Celtic Sea (panel A and B) and the North Sea (panel C and D) in function of the distance of the sampling locations to the coast: 0km (panel A and C) and 200 km (panel B and D). Polychlorinated biphenyls (PCBs) data included concentrations of seven congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) which were compared with the OSPAR consensus-based sediment effect concentration (0.50 – 47.5 µg.kg dw^{-1}; green polygon) and the actual quality threshold (24.0 µg.kg dw^{-1}; solid line) inferred from MacDonald et al. (2000). The horizontal dashed line represents a risk quotient (RQ) value of 1.*

The bio-availability of contaminants plays a crucial role in their ecotoxicological effects (Burton 2002; Chapman and Mann 1999; Choueri et al. 2010; Montero et al., 2013), but is often neglected in ecotoxicological assessments (Wu et al., 2008). In the present research we showed that the increased total PCB sediment concentrations during phytoplankton bloom periods suggests -at least in theory- increased environmental risks (Fig. 4.1) as the PCB concentrations in sediments increase when phytoplankton blooms (Galban-Malagon et al., 2012; Everaert et al., submitted). However, those plankton-bound PCBs are not freely dissolved (Dachs et al., 2002). Therefore, environmental risk assessment should focus on the bio-available fraction of PCBs. In this context, a possible solution is the use of silicon rubber passive samplers in environmental risk assessment of organic chemicals (Huckins et al., 2006). Passive samplers result in more realistic outcomes than classic spot sampling as information is obtained on the bio-available fraction instead of total concentrations (Mills et al., 2012; Claessens, 2013). A second advantage is that passive samplers monitor all hydrophobic compounds for which the sampling material has affinity (Rusina et al., 2007; Wille et al., 2011), and not only well-known substances. The latter a positive because the marine environment typically contains much more chemicals than those available in monitoring data sets (Echeveste et al., 2010) and evidence is growing for the potential impacts of emerging substances which are not routinely monitored and assessed (Hutchinson et al., 2013; Gaw et al., 2014). A potential weakness, however, is that the passive samplers function as a black-box i.e. when observing a certain ecotoxicological effect it is not possible to relate this back to an individual substance, but only to the entire chemical mixture present on the

passive sampler. Overall, passive samplers can serve as direct link between monitoring and toxicity tests and are a promising way forward to better integrate monitoring and environmental risk assessment (Monteyne et al., 2013; Claessens, 2013).

Although in this chapter RQs are based on published EAC (OSPAR, 1998), the authors would like to note that some nuances are required before taking overall conclusions. As mentioned in one of the earlier paragraphs, the EAC used in the present research have, although often used in environmental management and assessment studies, not recently been updated. The basis of the EAC, being a single-species short-term sediment toxicity test (Plesha et al., 1988) and a non-peer-reviewed summary of the effects of PCBs on mink (Leonards et al., 1994), need revision. Since RQs are critically depending on the scientific robustness of the EAC, there is need for a review of these criteria and for careful interpretation of the results presented in this chapter.

4.4 Conclusion

The total sum of seven marker polychlorinated biphenyls ($\sum PCB_7$) in sediment at open sea are currently below 1.00 µg.kg dw^{-1} and were significantly lower than $\sum PCB_7$ in coastal waters. Risk quotients (RQs) at open sea remained below 1 within the investigated timeframe, but closer to the coast a potential ecotoxicological risk is suggested due to RQs above 1. PCB concentrations in mussels are decreasing within the investigated timeframe. Although in the Celtic Sea PCB concentrations remain below the prescribed threshold concentrations, PCB concentration in mussels in the North Sea may pose a potential ecotoxicological risk. Concentrations of PCBs in sediment (respectively mussels) were higher (respectively lower) between November and March than during spring and autumn. Our results demonstrate that the spatiotemporal variability in concentrations of PCBs should be considered in future environmental risk assessments as it may considerably alter conclusions taken.

5

Realistic environmental mixtures of organic contaminants do not alter growth of a marine diatom

Redrafted from:
Gert Everaert, Frederik De Laender, Michiel Claessens, Jan M. Baert, Els Monteyne, Patrick Roose, Peter L.M. Goethals, Colin R. Janssen. Realistic environmental mixtures of organic contaminants do not alter growth of the marine diatom *Phaeodactylum tricornutum*. Environmental Pollution. Under review.

Abstract

In the present paper it is determined whether a realistic environmental mixture of organic chemicals affects the growth dynamics of marine phytoplankton and how this effect compares to the effect of temperature, light regime and nutrient conditions. To do so, we examine the relative contribution of organic chemicals to the growth of a marine diatom, *Phaeodactylum tricornutum*. To this end, the specific growth rate of *P. tricornutum* was studied at 24h intervals in a 72h algal growth inhibition test using a full factorial design with three nutrient regimes, two test temperatures, three light intensities and three chemical exposures. Passive samplers were used to achieve exposure to realistic mixtures of organic chemicals close to natural concentrations. Nutrient regime, temperature and time interval (24, 48 and 72h) explained about 85% of the observed variability in the experimental data. Organic chemicals did not affect the intrinsic growth rate of *P. tricornutum* in a 72h growth inhibition test. Overall, based on an algal growth inhibition test with *P. tricornutum*, being a relative sensitive marine diatom, it is suggested that the ambient concentrations of organic chemicals present in Belgian marine waters are not likely to affect the growth of marine diatoms.

5.1 Introduction

A complex mixture of organic chemicals is present in the Belgian coastal and estuarine waters (Wille et al., 2011; Monteyne et al., 2013) of which the ecotoxicological risk is poorly understood (Claessens et al., 2013). Notwithstanding their low freely dissolved concentrations (Ghekiere et al., 2013), mixtures of organic chemicals can cause ecotoxicological effects in marine ecosystems (e.g. Echeveste et al., 2011; Poulsen et al., 2012) due to their persistency and bio-accumulative potential (Jones and De Voogt, 1999). Current polychlorinated biphenyls (PCBs) concentrations, for example, may still exceed quality thresholds despite their ban in the 1970s (Ghekiere et al., 2013). Especially in coastal and estuarine environments, concentrations of organic chemicals may exceed environmental quality standards (Ghekiere et al., 2013). Therefore, organic chemicals can impose risks to marine ecosystems (Halpern et al., 2008; Dachs and Méjanelle, 2010), but their ecotoxicological effect is not yet quantified (Rockström et al., 2009).

Phytoplankton is the basis of the pelagic food chain (Siegel and Franz, 2010), and alterations in the phytoplankton community can affect the entire ecosystem (Burkiewicz et al., 2005). An improved understanding of the ecotoxicological effects of organic chemicals on phytoplankton species is therefore needed. To date, ecotoxicological data are often based on single-chemical tests, and knowledge on the mixture toxicity effect of organic chemicals is limited to simple mixtures. Furthermore, it is well established that test temperature (e.g. Vieira and Guilhermino, 2012) and nutrient regimes (e.g. Kong et al., 2010) alter the ecotoxicological effects of organic chemicals in experimental assessments. However, to date, few studies (e.g. Sjollema et al., 2014) examined the combined effects of realistic environmental mixtures of organic chemicals and test conditions on the growth of marine phytoplankton. Hence, current ecotoxicological data may lack environmental realism.
Silicone rubber passive samplers, such as polydimethylsiloxane (PDMS) sheets, are increasingly used in environmental monitoring and assessment for their sampling and dosing ability (Lohmann and Muir, 2010). In passive sampling, PDMS sheets are deployed in an aquatic environment and accumulate hydrophobic chemicals (Vrana et al., 2005; Rusina et al., 2007). After deployment, accumulated chemicals can be extracted from the sheets to analyse the chemical composition of the sampling site (Emelogu et al., 2013; Monteyne et al., 2013). In passive dosing, PDMS sheets establish constant exposure concentrations (Claessens, 2013). Recently, this technique has been used in ecotoxicological research for chemicals with limited water-solubility (Claessens, 2013; Emegolu et al., 2013; Booji et al., 2013). To do so, sheets are first loaded with a mixture of organic chemicals, which can be done either passively (deployment in a contaminated aquatic environment) or actively (spiking). Next, sheets are added to a test medium where they release organic chemicals until equilibrium, continuously replenishing losses due to volatilization and adsorption (Othman et al., 2012).

The objective of the present paper is to determine whether a realistic environmental mixture of organic chemicals affect the growth dynamics of marine phytoplankton and how this effect compares to the effect of temperature, light regime and nutrient conditions. To this end, we used passive samplers (PDMS sheets) that were deployed along the Belgian coast (December 2013 – March 2014). Next, we evaluated the effect of three nutrient regimes, two test

temperatures, three light intensities and three chemical exposures on the growth of the marine diatom *Phaeodactylum tricornutum* in a 72h growth inhibition test.

5.2 Materials and methods

5.2.1 Preparation and deployment of passive samplers

Polydimethylsiloxane (PDMS) sheets were used to expose diatoms to natural mixtures of organic chemicals at environmentally realistic concentrations. PDMS sheets had a surface area of ca. 50 cm² (55 mm × 90 mm), a thickness of 0.5 mm and a mean mass of 3.15 g. Prior to deployment, all sheets were pre-extracted for 100h in boiling ethyl acetate using a Soxhlet extractor as in Monteyne et al. (2013). The PDMS sheets were attached to stainless steel cages and deployed along the Belgian coast at sampling station MOW1 (51° N 21.644', 3° E 6.992') at a depth of 9m between 10 December 2013 and 27 March 2014. Details on the oceanographic characteristics at the sampling location are in Table C.1. After deployment, the PDMS sheets were transported in pre-cleaned closed glass containers at −20 °C, and bio-fouling was removed with soft tissue and deionised water.

5.2.2 Algal growth inhibition test

Phaeodactylum tricornutum Bohlin strain 1052/1A was obtained from the Culture Collection of Algae and Protozoa (Oban, United Kingdom). The toxicity of organic chemicals was studied in a 72h growth inhibition test (ISO 10253) using a full factorial design. In the study design following factors were used: three nutrient regimes (14 µmol P L^{-1} & 588 µmol N L^{-1}; 2.8 µmol P L^{-1} & 120 µmol N L^{-1} and 0.7 µmol P L^{-1} & 30 µmol N L^{-1}), two test temperatures (16°C and 23°C), three light intensities (10,000 lux; 20,000 lux and 25,000 lux) and three chemical exposures, i.e. a blank control (containing no PDMS sheets), a growth control (containing unloaded PDMS sheets) and the actual chemical exposure (containing loaded PDMS sheets). Tree replicates were provided for each unique test condition.

P. tricornutum was maintained in the laboratory according to protocol ISO 10253 (ISO, 2006). Prior to the start of the growth inhibition test, two precultures were grown at both test temperature used in the growth inhibition test (i.e. 16°C and 23°C). Precultures were inoculated with an initial cell concentration of $5.0 * 10^4$ cells mL^{-1}.

Erlenmeyer flasks (100mL) were filled with 50 mL of growth medium (ISO 10253), and PDMS sheets (cut in four equal pieces) were added. After a two-day equilibrium period, each flask was inoculated with $1.0 * 10^4$ cells mL^{-1} of the exponentially growing *P. tricornutum* precultures and incubated at the prescribed test temperature and light intensities. Test flaks were shaken manually twice a day. The algal cell density was measured after 24h, 48h and 72h in each test flask using an electronic particle counter (Coulter Counter model DN, Harpenden, Herts, UK). The pH of the test medium was measured at the start and end of the test period (Table C.2). All glassware used in the algal growth inhibition test was sterilized in an autoclave at 121 °C for 40 min.

5.2.3 Statistical analysis

The specific growth rate (µ) was calculated according to protocol ISO 10253 (ISO, 2006):

$$\mu = \frac{\ln(N_L) - \ln(N_0)}{t_L - t_0} \qquad \text{(Eq. 5.1)}$$

with t_0 the moment of inoculation (day 0), t_L the moment of sampling (day 1, 2 or 3), N_0 the inoculated cell density at t_0 (1,0 * 10^4 cells.mL^{-1}) and N_L the measured cell density at t_L (cells.mL^{-1}). We used a linear regression model to check for differences in the specific growth rates between the full-factorial explored test conditions. The linear regression model, with Y_i the response variable and X_i the M covariates, was defined as:

$$Y_i = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \ldots + \beta_M X_{Mi} + \varepsilon_i \qquad \text{(Eq. 5.2)}$$

where the parameters α and β_M represent the intercept and the M slopes of the corresponding covariates (Zuur et al., 2009). The residuals ε_i capture the unexplained variation in the data.
The initial model included the day of sampling of the test flasks (further referred to as "sampling day"), the nutrient regime (three conditions), the test temperature (two conditions), the light intensity (three conditions) and the chemical exposure to organic contaminants through PDMS sheets (three conditions) as covariates. In addition to these main effects, we also included two way-interactions between the sampling day and the test conditions (Table 5.1). We started from the initial model and implemented a hierarchical backward elimination model selection method as in Everaert et al. (2014). The Akaike information criterion (AIC) weighed the model fit against the model complexity (Zuur et al., 2009) and was the primary criterion to select the model with the best fit. The lower the AIC, the better the model fit (Zuur et al., 2009). For each model configuration, we used the F-statistic and its associated p-value to asses which covariates contributed to the model at a 5% level of significance (Zuur et al., 2009). Residual plots showed that a quadratic term for the moment of sampling was required (Fig. C.6; Table C.3). The contribution of each covariate to the model fit was quantified by relative comparison of the sum of squared errors (SSE) of a model including a particular covariate with the SSE of a model not including this covariate:

$$\%\ \text{Variability explained by a covariate} = \frac{SSE_i - SSE_j}{SSE_i} * 100 \qquad \text{(Eq. 5.3)}$$

with SSE_j the sum of squared errors of model *j* (including the covariate) and SSE_i the sum of squared errors in model *i* (without the covariate). In a final step, we extended the selected model, that was obtained after the hierarchical backward elimination model selection method, with two-way interaction terms between the chemical exposure and the other test conditions (i.e. nutrient regime, test temperature and light intensity). We assessed the contribution of these interaction terms to the model fit and quantified the AIC of the model found.

5.2.4 Chemical analysis

Following the algal growth inhibition test, we quantified freely dissolved concentrations of fifteen polycyclic aromatic hydrocarbons (PAHs) and seven polychlorinated biphenyls (PCBs) in the erlenmeyer flasks (Table C.4). We randomly subdivided test flasks of the blank control (containing no PDMS sheets) in four groups, test flasks of the growth control

(containing unloaded PDMS sheets) in three groups and test flasks of the actual chemical exposure (containing loaded PDMS sheets) in three groups. By doing so, in total ten groups of test flasks were randomly created. Next, test flasks within each group were pooled to obtain ten volumes of about 1L in which we quantified freely dissolved concentrations of the PAHs and PCBs. To do so, for each volume, internal standards (deuterated analogues of parent PAH compounds (VWR) and PCB congeners 14, 112, 143, 155 and 204 (Sigma-Aldrich)) were added. Volumes were extracted three times with dichloromethane. The extract was dried on Na_2SO_4 and subsequently concentrated to about 0.2 mL using a rotary evaporator with N_2 cooling. Anthracene-d10 (VWR) was added as recovery standard. The extracts were analysed with GC/MS (Trace GC and Trace DSQ, Thermo Electron Cooperation). All solvents used were of purity suitable for organic residue analysis.

Table 5.1 *Summary of the hierarchical backward elimination model selection method. Seven different model configurations were tested and for each configuration we reported the Akaike information criterion (AIC), the adjusted correlation coefficient (R^2) and the variability explained by the covariates that were included. The AIC was the primary criterion to select the model configuration with the best model fit. Covariates considered were: the moment of sampling (DAY), the nutrient regime (NUT), the test temperature (TEM), the chemical exposure (ORC) and the light intensity (LIG). If appropriate quadratic effects (²) and interaction terms (:) were included. For each model configuration residual diagnostics are shown in supportive information.*

Model	Model configuration	AIC	Adjusted R^2	Variability explained	Residual plots shown in
1	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM + (DAY + DAY²) * LIG + (DAY + DAY²) * ORC	-387.9	0.77	88.1	Fig. C.1
2	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM + (DAY + DAY²) * LIG	-392.4	0.77	87.0	Fig. C.2
3	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM	-392.3	0.76	85.2	Fig. C.3
4	(DAY + DAY²) * NUT	-309.9	0.59	66.6	Fig. C.4
5	DAY + DAY²	-192.8	0.12	12.8	Fig. C.5
6	DAY	-191.2	0.11	10.7	Fig. C.6
7	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM + (DAY + DAY²) * LIG + ORC:NUT + ORC:TEM + ORC:LIG	-397.0	0.79	90.8	Fig. C.7

5.3 Results and Discussion

5.3.1 Algal growth inhibition test

Freely dissolved concentrations of organic chemicals present along the Belgian coast (Table C.4) did not exert a direct effect on the specific growth rate of the marine diatom *Phaeodactylum tricornutum* in a 72h growth inhibition test (Table 5.1). We tested the effect at two test temperatures, three nutrients regimes, three light intensities and three chemical exposures on the growth of *P. tricornutum.* We found that the day of sampling of the test flasks ("sampling day") and the nutrient regime explained about 65% of the observed variability in the experimental data (model 4; Table 5.1). Integration of test temperature as an extra covariate in the model increased the explained variability to about 85% (model 3; Table 5.1 and Fig. 5.1). Including a main effect for chemical exposure increased the Akaike information criterion (AIC) to -387.9 (model 1; Table 5.1). The explained variability owing to chemical exposure was limited to 1.1% (88.1% - 87.0%; Table 5.1) and not significant ($p = 0.126$; Table C.3). Putting this in perspective to the inherent methodological variability estimated at maximum 7% (ISO, 2006), indicates that chemical exposure had no main effect on the growth of *P. tricornutum*. The latter is confirmed when statistically comparing the fit of model 1 (including chemical exposure) with the fit of model 2 (excluding chemical exposure), i.e. model 1 is equal to model 2 (F-test; $p = 0.009$). Furthermore, light intensities had a limited (1.8%; Table 5.1) but significant ($p = 0.011$; Table C.3) main effect on the specific growth rate of *P. tricornutum*. The variability explained by including a main effect for temperature (about 20%; Table 5.1), is illustrated in Figure C.8. This figure shows the non-log-transformed algal densities in the first 72h in the algal growth inhibition test. Note that the positive effect of an increased test temperature on the cell density of *P. tricornutum* is mainly observed under the highest nutrient concentrations (red boxplots in Figure C.8). Indeed, when the amount of nutrients available is lower (green and blue boxplots) the positive effect of the test temperature on the algal density decreases (Fig. C.8). Overall, about 85% of the variability in the specific growth rate of the marine diatom was explained by the sampling day, the nutrient regime and the test temperature (Fig. 5.1). No main effects of chemicals on the specific growth rate of *P. tricornutum* were found (Table 5.1 and C.3).

In addition to the main effects, we tested whether adding an interaction term between the chemical exposure and the nutrient regime, between the chemical exposure and the test temperature and between the chemical exposure and the light intensities increased the model fit (model 7; Table 5.1). Inclusion of these interaction terms improved the model ($p = 0.25$; $p = 0.08$ and $p = 0.04$, respectively) (Table C.3) as the AIC decreased from -392.4 (model configuration 2) to -397.0 (model 7; Table 5.1). However, note that the gain in model fit was limited to 3.8% (90.8% - 87.0%) and was mainly attributed to the significant interaction between the chemical exposure and light intensities ($p = 0.04$; Table C.3). Interaction effects between the light conditions and the toxicity of organic chemicals were earlier reported by Lyons et al. (2002) and Echeveste et al. (2011). Regarding the two other interaction effects, Kong et al. (2010) reported that a single-cell green alga *Chlorella vulgaris* was more sensitive to organic chemicals under nutrient-enriched growth conditions. However, we did not detect an interaction effect between chemical exposure and nutrients in our experiment ($p = 0.25$; Table 5.1 and C.3). Consistent with the findings of Kong et al. (2010), we did find that a

diminishing nutrient supply decreased the specific growth rate (Fig. 5.2). Next to nutrient availability, test temperature may also alter the toxicity of organic chemicals (Saeed et al., 1998; Vieira and Guilhermino, 2012). However, as for nutrients, the test temperature did not change the ecotoxicological effect of organic chemicals ($p = 0.08$; Table 5.1 and C.3). Overall, freely dissolved organic chemicals sampled along the Belgian coast had no main effect and a limited interaction effect with light conditions on the growth of *P. tricornutum* under laboratory conditions.

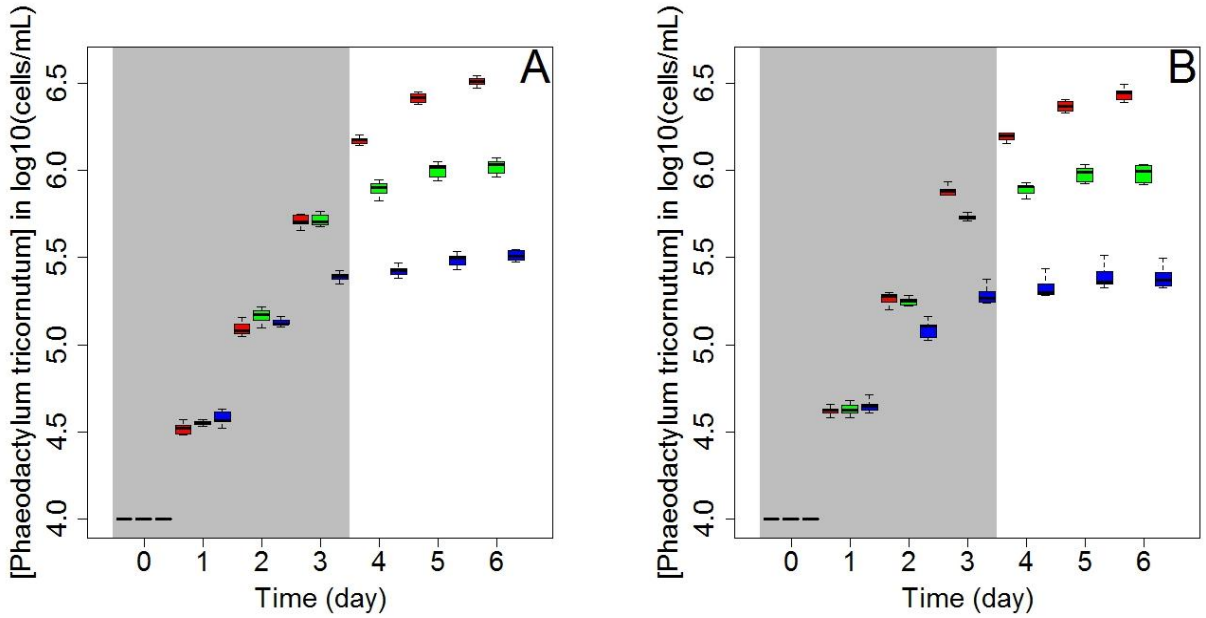


Figure 5.1 *Two-panel boxplot illustrating the evolution of Phaeodactylum tricornutum concentrations (log10 cells.mL^{-1}) in the algal growth inhibition test over time for two test temperatures: 16°C (A) and 23°C (B). For each test temperature, three different nutrient regimes are plotted: 14 µmol P.L^{-1} & 588 µmol N.L^{-1} (red), 2.8 µmol P.L^{-1} & 120 µmol N.L^{-1} (green) and 0.7 µmol P.L^{-1} & 30 µmol N.L^{-1}(blue). The grey boxes delimit the experimental data after 72h on which the statistical analysis was based. Each single boxplot covers three chemical exposure and three light intensities.*

Although we assessed the ecotoxicological effect of organic contaminants to only one marine algal species, it is remarkable that results obtained corroborate those of Everaert et al. (2015). Using a mechanistic ecosystem model (see further, chapter VI) Everaert et al. (2015) estimated an organic chemical-induced growth effect on marine phytoplankton in Belgian marine waters of about 1%, equal to the estimated contribution of the chemical exposure to the algal growth inhibition data, i.e. 1.1% (88.1% - 87.0%; Table 5.1). These results seem contrasting with findings of Emegolu et al. (2013) and Echeveste et al. (2010) who reported that a natural mixture of the organic chemicals can inhibit the marine primary production. However, methodologies differed substantially from our paper. Both Emegolu et al. (2013) and Echeveste et al. (2010) tested concentrations which are higher than natural concentrations. Emelogu et al. (2013) found potential toxicity effects on the flagellate (*Diacronema lutheri*) in a 72h algal growth inhibition test using a passive sampler extract dilution series. Similarly, Echeveste et al. (2010) reported adverse ecotoxicological effects of natural mixtures of organic chemicals at 20 to 40-fold the concentrations found in the open

ocean. Here, we exposed *P. tricornutum* not only to natural mixtures of organic chemicals, but also to freely dissolved concentrations present in Belgian coastal waters at which we did not observe direct adverse ecotoxicological effects under laboratory conditions.

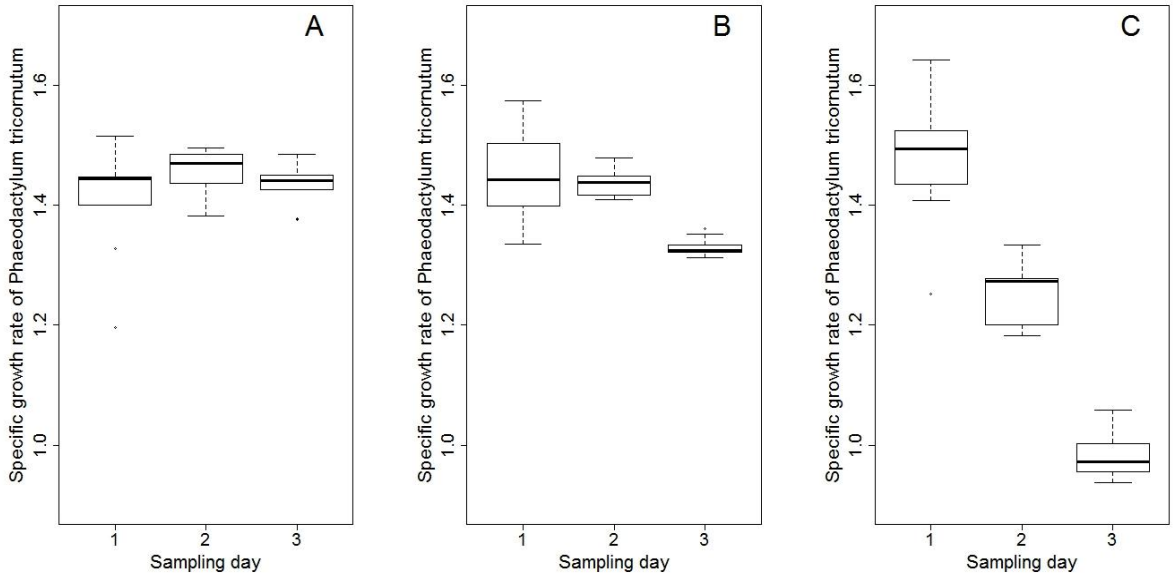


Figure 5.2 *Specific growth rate of Phaeodactylum tricornutum in the first 72h of a growth inhibition test at 23°C under three different nutrient regimes: 14 µmol P.L^{-1} & 588 µmol N.L^{-1} (A), 2.8 µmol P.L^{-1} & 120 µmol N.L^{-1} (B) and 0.7 µmol P.L^{-1} & 30 µmol N.L^{-1} (C). Under the highest nutrient regime (A) we observe that P. tricornutum continues to grow at the same rate for -at least- 72h. If less nutrients are available (B and C) the specific growth rate gradually slows down over time. Each single boxplot covers three chemical exposure and three light intensities.*

5.3.2 Chemical analysis

The total concentration of seven PCBs ($\sum_7$PCBs) was 6.8 ± 1.5 ng.L^{-1} and the total concentration of fifteen PAHs ($\sum_{15}$PAHs) was 39.7 ± 9.8 ng.L^{-1} (Table C.4), which is close to the range of concentrations reported by Monteyne et al. (2013) along the Belgian coast and by Emegolu et al. (2013) in Scottish estuaries (Table 5.2 and C.4). Note that the $\sum_7$PCBs and $\sum_{15}$PAHs reported here are lower than concentrations measured in harbours, but higher than those at open sea (Garcia-Flor et al., 2005; Josefsson et al., 2011). Along the Belgian coast, Monteyne et al. (2013) found that the background concentrations of $\sum_7$PCBs (2 pg.L^{-1}; Josefsson et al., 2011) were exceeded by a factor of 15 (open sea samples) and 1500 (harbour samples). In the present study, $\sum_7$PCBs exceeded those background concentrations by a factor 300. In addition, current $\sum_{15}$PAHs are a factor 20 higher than background levels for the North Sea (2 ng.L^{-1}; Harman et al., 2009) which is in the range reported by Monteyne et al. (2013), i.e. a factor 2 (open sea samples) to 85 (harbour samples). The latter indicates that our study site is neither a true harbour environment, nor represents open sea conditions. The study area in the present paper is, at least regarding concentrations of organic chemicals, a typical coastal water. Overall, freely dissolved concentrations of PCBs and PAHs found in the present paper are in accordance with those of other studies (Table 5.2). Hence, by using the polydimethylsiloxane (PDMS) sheets we exposed *P. tricornutum* to natural concentrations of

organic chemicals occurring in Belgian coastal and estuarine waters (Table 5.2 and C.4). In this context it is important to mention that the Belgian coastal and estuarine waters are subject to diffuse inputs of chemicals from industrial, agricultural and urban run-off (Voorspoels et al., 2003; Monteyne et al., 2013; Ghekiere et al., 2013). Therefore, next to PCBs and PAHs also other organic chemicals, such as pesticides, pharmaceutical or other hydrophobic (emerging) chemicals partitioned to the deployed PDMS sheets (Wille et al., 2010; Wille et al., 2011; Claessens et al., 2013, Emelogu et al., 2013). Most of these chemicals such as atrazine, diuron, isoproturon, salicylic acid and carbamazepine are detected at non-toxic concentrations (Wille et al., 2011). However recently, Sjollema et al. (2014) reported ecotoxicological effects of ambient concentrations of diuron on the phytoplankton community.

Table 5.2 *Comparison of concentrations of freely dissolved polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in remote waters, coastal and estuarine environments and in harbours. The reported ranges represent total concentrations (∑) of PCBs and PAHs.*

Location	No. congeners	Reported concentrations (ng.L^{-1})	Reference
Norway, inner Oslofjord	$\sum_{16}$PAHs	2.3 – 13.6	Schaanning et al. (2011)
Norway, oil platform	$\sum_{27}$PAHs	32 - 49	Harman et al. (2009)
Belgium, coastal harbours	$\sum_{15}$PAHs	3.9 - 170	Monteyne et al. (2013)
Scotland, Ythan estuary	$\sum_{40}$PAHs	69	Emelogu et al. (2013)
Scotland, Forth estuary	$\sum_{40}$PAHs	48.6 – 69.5	Emelogu et al. (2013)
Scotland, Forth estuary	$\sum_{16}$PAHs	20.4 – 26.1	Emelogu et al. (2013)
Atlantic Ocean	$\sum_{10}$PAHs	0.7 – 1.0	Nizzetto et al. (2008)
Belgium, coastal region	$\sum_{15}$PAHs	39.7 ± 9.8	Present study
Norway, inner Oslofjord	$\sum_{7}$PCBs	<0.01 - 0.20	Schaanning et al. (2011)
Australia, Sydney harbour	$\sum_{12}$PCBs	0.021 - 0.54	Roach et al. (2009)
Belgium, coastal harbours	$\sum_{14}$PCBs	0.03 – 3.10	Monteyne et al. (2013)
Scotland, Ythan estuary	$\sum_{7}$PCBs	0.01	Emelogu et al. (2013)
Scotland, Ythan estuary	$\sum_{32}$PCBs	0.02	Emelogu et al. (2013)
Scotland, Forth estuary	$\sum_{7}$PCBs	0.07 – 0.13	Emelogu et al. (2013)
Scotland, Forth estuary	$\sum_{32}$PCBs	0.16 – 0.30	Emelogu et al. (2013)
China, Pearl river delta	$\sum_{20}$PCBs	0.12 – 1.47	Guan et al. (2009)
Atlantic Ocean	$\sum_{7}$PCBs	0.071 – 1.7 (pg.L^{-1})	Gioia et al. (2008)
Spain , Barcelona harbor	$\sum_{41}$PCBs	0.295 – 7.617	Garcia-Flor et al. (2005)
Spain, Banyuls harbor	$\sum_{41}$PCBs	7.137 – 15.765	Garcia-Flor et al. (2005)
USA, Hudson estuary	$\sum_{90}$PCBs	0.370 – 1.600	Yan et al. (2008)
Belgium, coastal region	$\sum_{7}$PCBs	6.8 ± 1.5	Present study

Although $\sum_{7}$PCBs exceeded the environmental quality standards (2 ng.L^{-1}, Ghekiere et al., 2013), we did not observe adverse ecotoxicological effects in an algal growth inhibition test using natural concentrations of realistic mixtures of organic chemicals. Concentrations of the individual PAHs remained below the environmental quality standards (Table C.4). Environmental quality standards aim to protect an entire ecosystem and not just one particular (group of) species. In this context, *P. tricornutum* is often used in ecotoxicological research

(e.g. Okay et al., 2002; Wang et al., 2008) as it is: (1) a species that is prescribed by an internationally accepted toxicity test procedure (ISO 10253) and; (2) a relatively pollution sensitive species within the phytoplankton community. Unfortunately, only few studies compare the sensitivity to chemicals of marine phytoplankton species and none of them relates to PCBs. Claessens (2013) found that the sensitivity of *P. tricornutum* to pharmaceuticals was substance-specific. For some substances, such as propranolol and trimethoprim, *P. tricornutum* reported the lowest effect concentrations (i.e. *P. tricornutum* being the most sensitive species). For other substances such as salicylic acid and carbamazepine, *P. tricornutum* showed low sensitivity compared to other phytoplankton species. The comparative study Pavlic et al. (2005) tested the sensitivity of two marine diatom (*Skeletonema costatum* and *P. tricornutum*) and two freshwater green microalgae (*Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus*) to personal care products and concluded that the marine diatoms were more sensitive than freshwater green algae. Amongst both marine diatoms tested, *S. costatum* was most sensitive. Although it would allow more general conclusions, constructing species sensitivity distributions is difficult as too little toxicity data is available in open literature.

The main conclusion of the present study is that POPs present in Belgian marine waters did not affect the growth of *P. tricornutum.* Caution is needed when extrapolating these results to field conditions. In the present research, results were obtained under laboratory controlled conditions with one single species. In reality however, multiple species interact in the marine environment. Hence a small deviance (few %) of the algal growth rate of a particular species due to the presence of POPs may have serious implications for the entire phytoplankton community as these deviance may already lead to a competitive (dis)advantage over other species. As such, although the present research yields interesting quantifications, patterns and trends for one single diatom, it is suggested to assess the validity of the results in a mesocosm experiment (including multiple species and different trophic levels) or under field conditions before extrapolating these results to other diatoms and other groups of phytoplankton species. Although the present research suggests that natural concentrations of realistic mixtures of organic chemicals present in Belgian coastal waters do not directly affect the growth of the marine diatom *P. tricornutum* (Fig. 5.1 and Table 5.1), no ecotoxicological conclusions can be drawn for other plankton groups or higher trophic levels.

5.4 Conclusion

We performed an algal growth inhibition test under different environmental conditions (nutrients, temperature and light intensities). The aim of this design, i.e. environmental conditions x POP stress was to assess the contribution of the former variables to the growth dynamics of the marine diatom relatively to that of the potential adverse effects of organic chemicals. To do so, passive samplers were used to expose a marine diatom to a realistic mixture of organic chemicals present along the Belgian coast. Although $\sum_7$PCBs exceeded the environmental quality standards (2 ng.L^{-1}), we did not observe adverse ecotoxicological effects in a 72h algal growth inhibition test. We calculated the explained variability related to chemical exposure to be about 1%, but the main effect of the organic chemicals on the growth

of the marine diatom was not significant ($p = 0.126$). Natural drivers such as nutrients, temperature and light availability, explaining about 85% of the observed variability are more important drivers of the growth of a marine diatom (i.e. *P. tricornutum*) than the mixture of organic pollutants present in Belgian marine waters.

6

Relative contribution of organic chemicals to the growth dynamics of marine primary production based on multidecadal field data

Redrafted from:
Gert Everaert, Frederik De Laender, Peter L.M. Goethals, Colin R. Janssen (2015). Relative contribution of persistent organic pollutants to marine phytoplankton biomass dynamics in the North Sea and Kattegat region. Chemosphere. DOI: 10.1016/j.chemosphere.2015.03.084.

Abstract

In this paper, we use concentrations of persistent organic pollutants (POPs) and of chlorophyll a to infer POP-induced effects on marine primary production in the Kattegat and the North Sea between the 1990s and the 2000s. To do so, we modelled phytoplankton dynamics using four classical drivers (light and nutrient availability, temperature and zooplankton grazing) and tested whether extending this model with a POP-induced phytoplankton growth limitation term improved model fit to observed chlorophyll a concentrations. Including monitored concentrations of PCBs and pesticides did not lead to a better model fit, suggesting that POP-induced growth limitation of marine phytoplankton in the North Sea and the Kattegat is small compared to the limitations caused by the classical drivers. In an attempt to more fully represent the multitude of POPs in the marine environment, the monitored concentrations were multiplied with a factor 10 and 100. Under these two configurations, region-specific contributions of POPs in the phytoplankton growth limitation were found. The model-based contribution of POPs to phytoplankton growth limitation was ca. 1% in Belgian marine waters, but in the Kattegat POPs explained ca. 10% of the phytoplankton growth limitation. These results suggest that there are regional differences in the contribution of POPs to the phytoplankton growth limitation.

6.1 Introduction

The marine environment is the main receptor of persistent organic pollutants (POPs), even in areas distant from direct sources (Dachs et al., 2002). International conventions, such as the United Nations Environment Programme Stockholm convention on POPs, aim to control primary POP sources, but POPs are still present in many ecosystems today (Pozo et al., 2009) and may exceed environmental quality thresholds. Therefore, ecotoxicological effects of POPs need to be better understood and quantified (Rockstrom et al., 2009). Phytoplankton growth is a measure of marine ecosystem health (Siegel and Franz, 2010). Although marine phytoplankton is responsible for ca. 50% of the global net primary production (Falkowski and Raven, 2007), the impact of POPs on marine phytoplankton is poorly understood (Echeveste et al., 2010). The limited data available suggest that, under laboratory conditions, POPs decrease phytoplankton growth (Magnusson et al., 2008). However, the multitude of POPs present in the marine environment outnumbers the well-known POPs that are often used in experimental ecotoxicological studies (Echeveste et al., 2010). To test more ecologically relevant POP mixtures, Echeveste et al. (2010) exposed marine phytoplankton to natural mixtures of organic chemicals and observed toxic effects of POPs at concentrations 20–40 times higher than those found in the open ocean. In the same study, the authors concluded that the effect of complex mixtures of organic chemicals on marine phytoplankton can exceed the toxicity expected for a single pollutant by a factor of 1000. Hence it is recommended to further evaluate the effects of natural POP mixtures on marine primary production not only based on laboratory experiments (which may underestimate their toxicity), but also under field conditions (Echeveste et al., 2010) and in modelling studies (Law et al., 2010).

Human-induced changes in the marine environment need to be better understood and quantified. In this respect, the ability to separate contaminant effects from the effects of classical drivers is essential. For example, the effects of POPs on marine phytoplankton production may remain unnoticed since light, nutrients and temperature dynamics are primary determinants of phytoplankton biomass dynamics (Boyd and Hutchins, 2012). Being the energy source for photosynthesis, light availability drives most marine ecosystems and regional and seasonal changes of light regimes have important implications for phytoplankton production (Letelier et al., 2004). Next to light, phytoplankton dynamics are also determined by seasonal nutrient cycling which is, in temperate regions, characterized by high concentrations in winter, a progressive decrease during the spring and summer period, and a replenishment of nutrient levels in late summer and autumn (Arndt et al., 2011). Overall, light, nutrients and temperature explain 65% of the phytoplankton biomass dynamics (Irwin and Finkel, 2008). In addition, also zooplankton grazing may control phytoplankton biomass (Ruzicka et al., 2011). Given the effects of light, temperature, nutrients and zooplankton grazing, it is unclear whether and how much POPs affect phytoplankton dynamics.

In this study we test to what extent POPs may have impacted on phytoplankton dynamics in coastal and estuarine ecosystems, relative to four classical drivers: light and nutrient availability, temperature, and zooplankton grazing. To this end, we select two regions, one in the Belgian coastal zone (southern part of the North Sea) and one in the Kattegat (strait between North Sea and Baltic Sea) as both have a long history of chemical pollution and are globally amongst the most intensively monitored seas (Roose et al., 2011). We expected

different POP effects between both regions, driven by dissimilar POP fingerprints and concentrations (Biselli et al., 2005). For each region, we first tested if a classical nutrient-phytoplankton-zooplankton (NPZ) model, including the four classical drivers, fitted the observed phytoplankton biomass dynamics better when extending this model with POP-induced phytoplankton growth limitation. Next, we quantified the POP-induced growth limitation relative to the total growth limitation of all drivers in the NPZ model.

6.2 Materials and methods

6.2.1 Data

Two study regions were selected, one in the Belgian coastal zone (BCZ) and one in the Kattegat region (Fig. D.1). The first study region is located in Belgian marine waters, 233 km² large and has a water depth of about 10 m. The second study is located at the Swedish west coast, is 1144 km² large and has a water depth between 10 m and 75 m. Observed physical-chemical and biological data were obtained from the Belgian Marine Data Centre (BMDC, http://www.mumm.ac.be/) for the BCZ and the International Council for the Exploration of the Sea (ICES, http://www.ices.dk/) for the Kattegat region (Tables D.1-D.4). The physical-chemical data included nutrient concentrations, chlorophyll a concentrations (used as a proxy for phytoplankton biomass) and water temperature measurements, were recorded at a depth of 3m (BCZ) and at the water surface (Kattegat) (Tables D.2 and D.4). The persistent organic pollutant (POP) data included the concentrations of seven polychlorinated biphenyls (PCBs; 28, 52, 101, 118, 138, 153, 180) and four pesticides (α-hexachlorocyclohexane, γ-hexachlorocyclohexane, p-p'-dichlorodiphenyltrichloroethane and hexachlorobenzene; Table D.1 and D.3) which were reported to be of concern in the studied ecosystems (Ghekiere et al., 2013). PCBs and pesticides were measured in sediment and in biota (liver of cod – *Gadus morhua*) for the BCZ and Kattegat, respectively. The organic carbon-water partition coefficient, the octanol-water partitioning coefficient, a 2% organic carbon content of the sediment (Zaborska et al., 2008) and the bioaccumulation factor per POP were used to convert the sediment concentrations (BCZ) and concentrations measured in cod (Kattegat) to the internal phytoplankton lipid weight concentrations (Fig. D.2). The bioaccumulation model of Hendriks et al. (2001) was used to estimate the bioaccumulation factors. A detailed description of the bioaccumulation model including the steps taken, the variables and parameterization used and substance-specific information is provided in Tables 1.1 and D.5.

6.2.2 Time trends from data

Additive models (Wood, 2006) were used to infer time trends of nutrient concentrations, water temperature and POP concentrations (Fig. 6.1). Covariates used in the additive models were time (as year and month) and the name of the substance under study. Additive models were already used by Everaert et al. (2014) to infer spatiotemporal trends in the marine environment. The Akaike information criterion and the distribution of the model residuals were used to assess the fit of the additive models (Fig. 6.2 and 6.3; Table D.6 and D.7) (Zuur et al., 2009). Furthermore, predictions were plotted against observations to visualize model fit (Fig. D.3 and D.4).

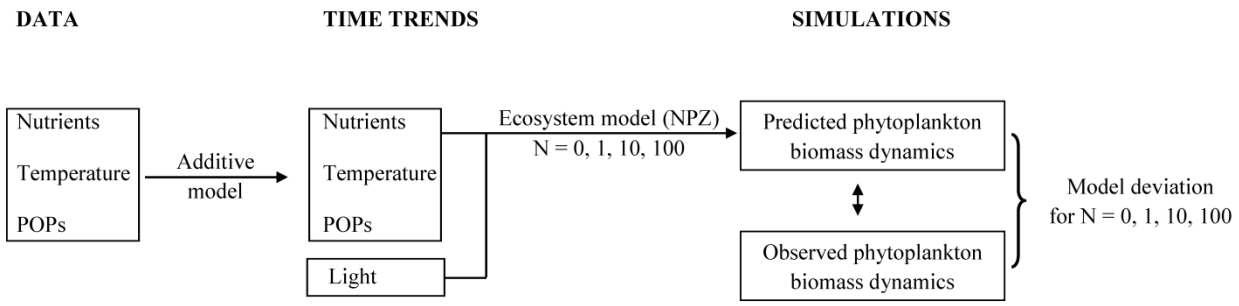


Figure 6.1 *Overview of the adopted strategy. Additive modelling was used to infer time trends for temperature, nutrients and persistent organic pollutant (POP) concentrations. Those time trends in combination with empirical light conditions were used to simulate chlorophyll a concentrations based on a nutrient-phytoplankton-zooplankton (NPZ) model. Four NPZ configurations were tested assuming: no POPs present (N = 0), monitored POP concentrations (N = 1), a ten-fold of the monitored POP concentrations (N = 10) and a hundred-fold of the monitored POP concentrations (N = 100). Finally, the simulated chlorophyll a concentrations were compared to observed data.*

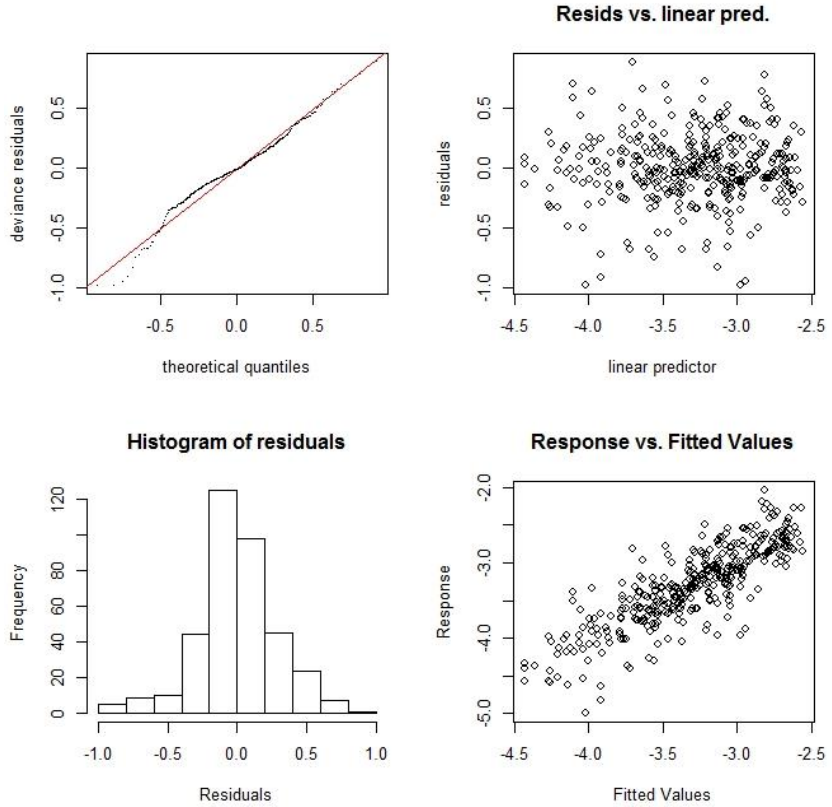


Figure 6.2 *Residual analysis of the additive model for the PCB and pesticide concentrations in the Belgian coastal zone. The QQ plot and the histogram are used to assess normality, and the residuals versus fitted values homogeneity. The response versus fitted values should ideally show a straight line.*

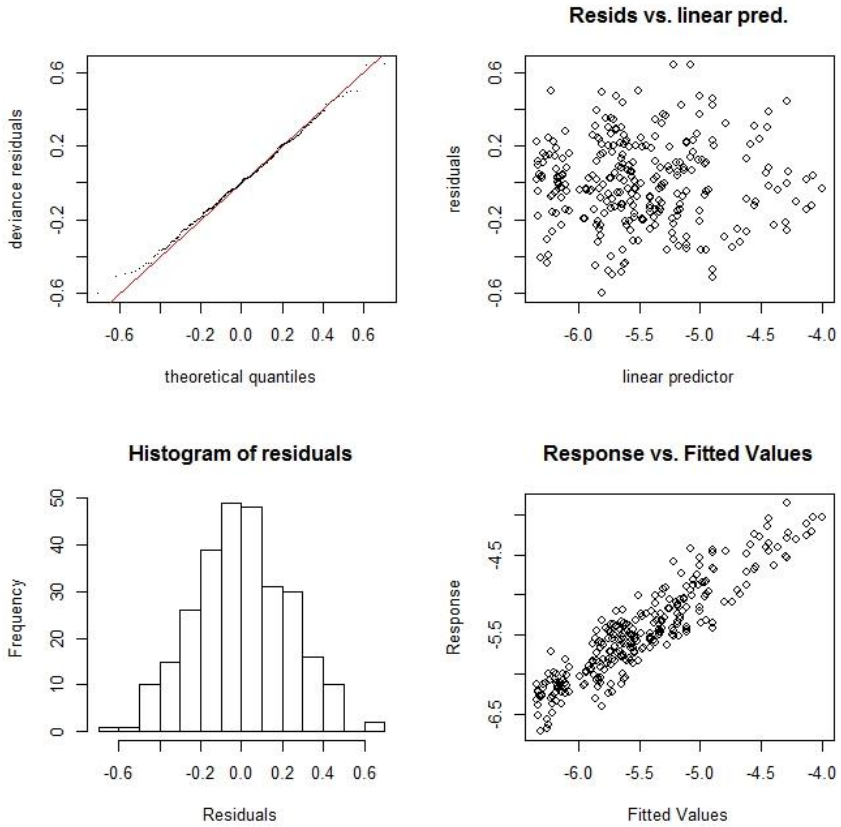


Figure 6.3 *Residual analysis of the additive model for the PCB and pesticide concentration in the Kattegat region. The QQ plot and the histogram are used to assess normality, and the residuals versus fitted values homogeneity. The response versus fitted values should ideally show a straight line.*

6.2.3 Model

The nutrient-phytoplankton-zooplankton (NPZ) model developed by Soetaert and Herman (2009) was used to simulate phytoplankton biomass dynamics based on five drivers: photosynthetically active radiation (PAR), dissolved inorganic nitrogen (DIN), phosphate (PO_4), water temperature and POPs (Irwin and Finkel, 2008; Ruzicka et al., 2011). The time trend of PAR was calculated as in Soetaert and Herman (2009) (Fig. D.5). PAR and nutrients (DIN and PO_4) were implemented in the NPZ model as saturating Michaelis-Menten equations (Arndt et al., 2011; Soetaert and Herman, 2009). A Michaelis-Menten term varies between one (no limitation) and zero (complete limitation of the phytoplankton growth) (Arndt et al., 2011). The influence of water temperature was calculated as in Thomann and Mueller (1987). The impact of POPs on the phytoplankton biomass dynamics (P_{max}; d^{-1}) (Eq. 6.1) was introduced as a logistic concentration-effect function (De Laender et al., 2008), based on the critical body burden (CBB) theory (McCarty et al., 1993) and was described as:

$$P_{max} = \frac{P_{max,0}}{1+\left(\frac{TOX}{CBB_{50,Pmax}}\right)^{slope}} \quad \text{(Eq. 6.1)}$$

With $P_{max,0}$ = the maximum photosynthetic rate at control (d^{-1}); slope = the slope of the concentration-effect function; TOX = the body burden of POPs (mmol.kg wet weight^{-1});

$CBB_{50,Pmax}$ = body residue concentration corresponding with a 50% reduction of the maximum photosynthetic rate (mmol.kg wet weight^{-1}).
The CBB is a body residue concentration that is relatively constant across species and narcotic compounds (Vanwezeland Opperhuizen, 1995) and varies between 2 and 8 mmol.kg wet weight^{-1} (McCarty et al., 1993). This makes the CBB useful to assess the effects of mixtures of non-specifically acting bioaccumulative chemicals (Escher et al., 2011). Based on concentration addition, the concentrations of seven PCBs and four pesticides were summed and assumed to represent the overall trend of PCBs and pesticides in the marine environment. However, the marine environment typically contains many more chemicals than those available in monitoring data sets (Echeveste et al., 2010). To account for this, the NPZ model was ran under four configurations. One model configuration (N = 0; '0TOX') assumed that no POPs were present, as such representing a baseline scenario. Configuration '1TOX' (N = 1) used the total phytoplankton body burden of the seven PCBs and four pesticides, i.e. the monitored POP concentration. Configurations 10TOX' (N = 10) and '100TOX' (N = 100) assumed the actual body burden was 10 and 100 times higher than the monitored concentration, respectively. As such, although being a rough assumption, the multiplication of the monitored concentrations of PCBs and pesticides by 10 and 100 serves as a surrogate for the unknown POPs present in the marine environment. For all model configurations, model parameters were sampled from uniform distributions reflecting the ranges reported in the literature (Table D.8; 10,000 iterations) (Soetaert and Herman, 2009). Each NPZ model configuration and parameterization resulted in simulated daily chlorophyll a concentrations for the entire sampling period.

6.2.4 Comparison between data and model

Daily simulations of chlorophyll a concentrations were averaged to monthly values and were compared to the corresponding observations (cfr. section on 'Data') similar as in Viaene et al. (2014). The model deviation (MD) was calculated as:

$$MD = \sum_{i=1}^{i=n} | \log_{10} \text{prediction}_i - \log_{10} \text{observation}_i | \qquad \text{(Eq. 6.2)}$$

With observation_i = the i^{th} observed monthly average chlorophyll a concentration; prediction_i = the simulated chlorophyll a concentration corresponding to observation_i; n = the total number of chlorophyll a observations. Averaging the daily NPZ–based chlorophyll a concentrations to monthly values was needed to compare the simulated chlorophyll a concentrations with the observed concentrations. Note that the NPZ–based chlorophyll a concentrations rely on nutrient and POP concentrations, and temperature and light information (Fig. 6.1) and do not included possible concentrations changes due to precipitation events, wind and current related mixing (O'Driscoll et al., 2013). The closer the simulated chlorophyll a concentrations are to the observed chlorophyll a concentrations (Fig. 6.4), the lower the MD. For each NPZ configuration, the ten percent best parameterizations (lowest MDs) were retained and boxplots and pairwise Wilcoxon rank-sum tests (with α = 0.05) were used to compare the distributions of the MDs of the four NPZ configurations. POPs significantly contributed to the phytoplankton biomass dynamics if the inclusion of POPs decreased the difference between model simulations and observations. Results of the

pairwise Wilcoxon rank-sum tests are summarized in Tables D.9 and D.10. Furthermore, based on the ten percent best parameterizations, a 95% confidence interval of the simulated chlorophyll a concentrations was calculated for the entire sampling period. The upper (97.5%) and lower limits (2.5%) of the confidence interval of the simulated chlorophyll a concentrations were plotted versus time and the observed chlorophyll a concentrations were also shown (Fig. 6.4).

6.2.5 Relative growth limitation for each driver

The relative limitation of each driver to the total monthly phytoplankton growth limitation was quantified per month, based on the ten percent best simulations per NPZ model configuration (Fig. 6.5). The relative limitations of all drivers were calculated using the corresponding limitation terms in the NPZ model (i.e. Michaelis Menten kinetics for PAR, DIN and P; empirical relation of Thomann and Mueller (1987) for temperature, logistic concentration-effect function for PCBs and pesticides). First, for each driver, the absolute limitation was calculated as 1 – the corresponding limitation term. Next, the relative contribution (in %) of each driver to the total limitation of the phytoplankton growth (the sum of all absolute limitations terms) was calculated.

6.3 Results and discussion

6.3.1 Nutrients and water temperature: time trends

In the Kattegat region, seasonal variations (Table D.7) were inferred for water temperature, phosphate, nitrate, nitrite and ammonium concentration, confirming the findings of Kronvang et al. (1993). Water temperatures attained maximum values in summer months and winter nutrient concentrations exceeded summer concentrations. Furthermore, water temperatures increased between 1990 and 2011 and phosphate and ammonium concentrations decreased (Table D.7), i.e. similar as the trends reported by Rydberg et al. (2006). In the Belgian coastal zone (BCZ), similar seasonal trends as in the Kattegat were found (Table D.6). Seasonal trends were similar to those described by Arndt et al. (2011) for the southern North Sea. Between 1991 and 2006, nitrite, ammonium and phosphate concentrations decreased. However, water temperature and nitrate concentration showed no long-term trend (Table D.6). In the BCZ, nutrient levels are mainly controlled by mixing of Atlantic waters with nutrient-rich freshwater discharges from the Scheldt, the Rhine/Meuse and the Seine estuary (Arndt et al., 2011; Lacroix et al., 2007).

6.3.2 Persistent organic pollutants: time trends

Overall, concentrations of PCBs and all pesticides decreased with time in both study regions (Fig. D.6). Between 1991 and 2006, decreasing time trends were inferred for the BCZ (Table D.6; Fig. D.7). In the Kattegat, concentrations of eight of the eleven persistent organic pollutants (POPs) decreased between 1990 and 2011, but concentrations of highly hydrophobic PCB congeners (CB138, CB153 and CB180) increased or were stable (Table D.7; Fig. D.8). Decreasing PCB and pesticide concentrations have been reported in many aquatic ecosystems (Everaert et al., 2014; Roose et al., 1998; Miller et al., 2013) which has mainly been attributed to their restricted use since the early 1970s. The deviating time trends

we found for CB138, CB153 and CB180 may be attributed to latitudinal fractionation of POPs, i.e. while volatile (high vapour pressure) POPs can travel over long distances, more hydrophobic (high Kow) stay closer to sources (Wania and Mackay, 1993). PCB congeners with relatively low mobility (e.g. CB138, CB153 and CB180) had elevated concentrations in the Kattegat. The fact that more hydrophobic substances show a higher tendency to accumulate in organic matter and to persist in fish liver may be an alternative explanation for the deviating time trends found for CB138, CB153 and CB180. The internal phytoplankton lipid normalized concentrations of PCBs and pesticides varied seasonally (Table D.6 and D.7) and were inversely related to the seasonal phytoplankton biomass dynamics. In spring, when phytoplankton biomass was high (Fig. 6.4), low dissolved aqueous POP concentrations were observed. Indeed, phytoplankton is known to accumulate POPs, as it accounts for most of the organic matter content in the marine photic water column (Dachs et al., 1999). By exporting plankton-bound POPs from the water column to the sediment (Dachs et al., 1999; Turchetto et al., 2012), phytoplankton blooms can deplete dissolved concentrations of POPs (Galban-Malagon et al., 2012). Seasonally variable POP concentrations have also been observed at higher trophic levels in the Arctic marine (Frantzen et al., 2011) and terrestrial environment (Bustnes et al., 2011). Increased hibernal internal POP concentrations have been attributed to food shortage and a faster metabolization of the accumulated fatty tissues in winter (Frantzen et al., 2011; Bustnes et al., 2011). Furthermore, also the loss of fatty tissue due to spawning activity in spring and early summer plays an important role in the internal POP concentrations (Lee et al., 1996).

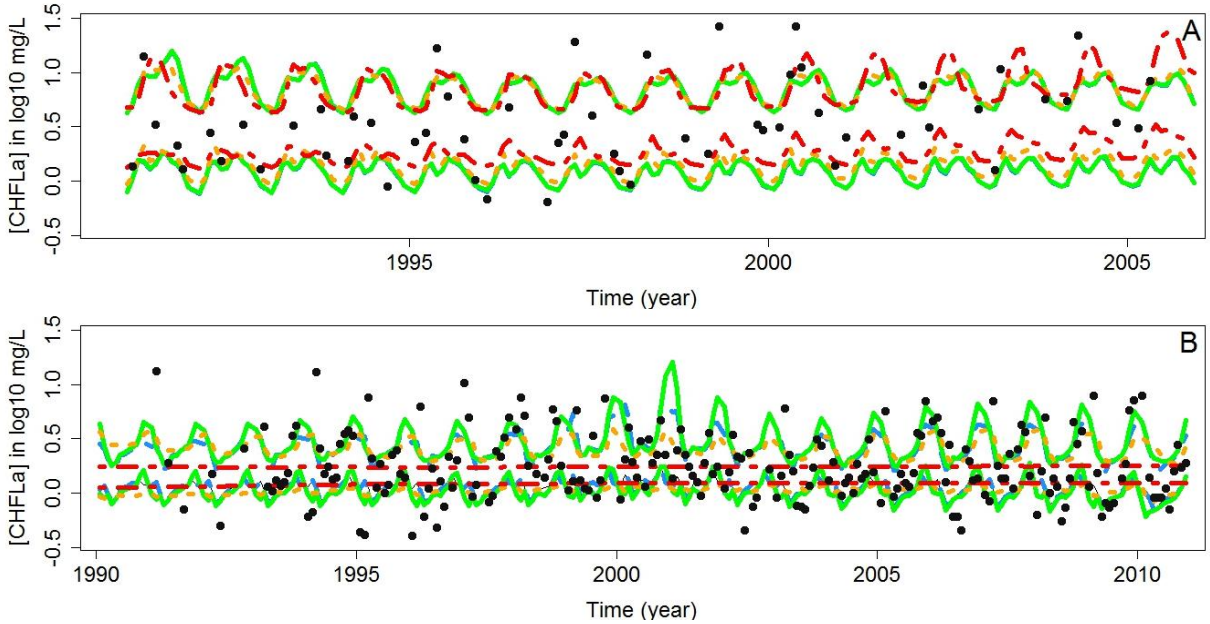


Figure 6.4 *Observed chlorophyll a concentrations (CHFLa, black dots) and the 2.5% - 97.5% confidence interval of the NPZ-based simulated chlorophyll a concentrations for four NPZ configurations tested assuming: no persistent organic pollutants (POPs) present (dashed, blue), (2) monitored POP concentrations (solid, green) and (3) ten-fold (dotted, orange) and (4) hundred-fold (dotdash, red) of the monitored POP concentrations in the Belgian coastal zone (A) and the Kattegat (B). In panel A is the blue line is hidden behind the green line as both model configurations make equal predictions.*

The sum of seven PCBs and four pesticides decreased between 1991 and 2006 in the BCZ (Fig. 6.6). However, due to the dominance of highly hydrophobic PCB congeners in the mixture, this sum increased between 1990 and 2011 in the Kattegat (Fig. 6.6). Similar as we have reported for the individual PCBs and pesticides, seasonal variation was observed in both study regions. In the BCZ, internal POP concentrations ranged from 0.05 mmol (summer) to 0.38 mmol per kg wet weight (winter). In the Kattegat, total POP concentrations ranged from 1.57 mmol (summer) to 5.74 mmol POPs per kg wet weight (winter) (Fig. 6.6).

6.3.3 Persistent organic pollutants: inferred effects

Including monitored concentrations of seven PCBs and four pesticides did not lead to better phytoplankton biomass simulations, suggesting that POP-induced growth limitation of marine phytoplankton in the BCZ (ca. 1%) and the Kattegat (ca. 10%) is relatively small compared to the limitations caused by the classical drivers (Fig. 6.5A and 6.5B). The influence of the classical drivers (e.g. photosynthetically active radiation (PAR), nutrient and temperature dynamics and zooplankton grazing) varied amongst study regions, i.e. they accounted for ca. 99% of the phytoplankton growth limitation in the BCZ (Fig. 6.5A) and for ca. 90% of the phytoplankton growth limitation in the Kattegat (Fig. 6.5B). Hence, the phytoplankton growth limitation was mainly determined by classical drivers. In the BCZ, zooplankton grazing was responsible for 50% of the phytoplankton growth limitation (Fig. 6.5A). The relative limitation due to temperature and light varied seasonally between 20% (summer) and 50% (winter). Nutrients played a less important role in the BCZ, which was also observed and concluded by Llope et al. (2009) and McQuatters-Gollop et al. (2007). The maximum limitation of nutrients to phytoplankton growth (30%) was obtained during the summer months (i.e. nutrient depletion). In the BCZ, the role of POPs in the phytoplankton growth limitation was never higher than 2%, varied seasonally, and decreased with time (Fig. 6.5A). In the Kattegat, zooplankton grazing and nutrients explained between 50% (winter) and 80% (summer) of the phytoplankton growth limitation (Fig. 6.5B). Rydberg et al. (2006) also found a co-variation between nutrient regimes and marine phytoplankton biomass dynamics in the same region; i.e. years of low nutrient supply coincided with low phytoplankton concentrations. The relative growth limitation due to temperature and light varied seasonally between 10% (summer) and 50% (winter). POPs explained between 10% (summer) to 20% (winter) of the phytoplankton growth limitation in the Kattegat (Fig. 6.5A and 6.5B).

To represent the multitude of POPs in the marine environment, the monitored concentrations were multiplied with a factor 10 (‘10TOX’) and 100 (‘100TOX’). Under these two configurations with elevated POP concentrations, regionally different POP-induced phytoplankton growth limitations were found (Fig. 6.7A and 6.7B). In the BCZ, multiplication of monitored POP concentrations with a factor 10 and 100 resulted in simulations that were closer to the observed phytoplankton biomass dynamics (Fig. 6.7A; Table D.9), suggesting that POPs influenced the phytoplankton growth in the BCZ. In the Kattegat however, simulated chlorophyll a concentrations assuming no POPs present (‘0TOX’) were closer to the observed chlorophyll a concentrations than the ecosystem model configuration with the monitored (‘1TOX’; $p < 2.2 * 10^{-16}$), a ten-fold (‘10TOX’; $p < 2.2 * 10^{-16}$) and a hundred-fold of the monitored POP concentrations (‘100TOX’; $p < 2.2 * 10^{-16}$; Fig. 6.7B; Table D.10).

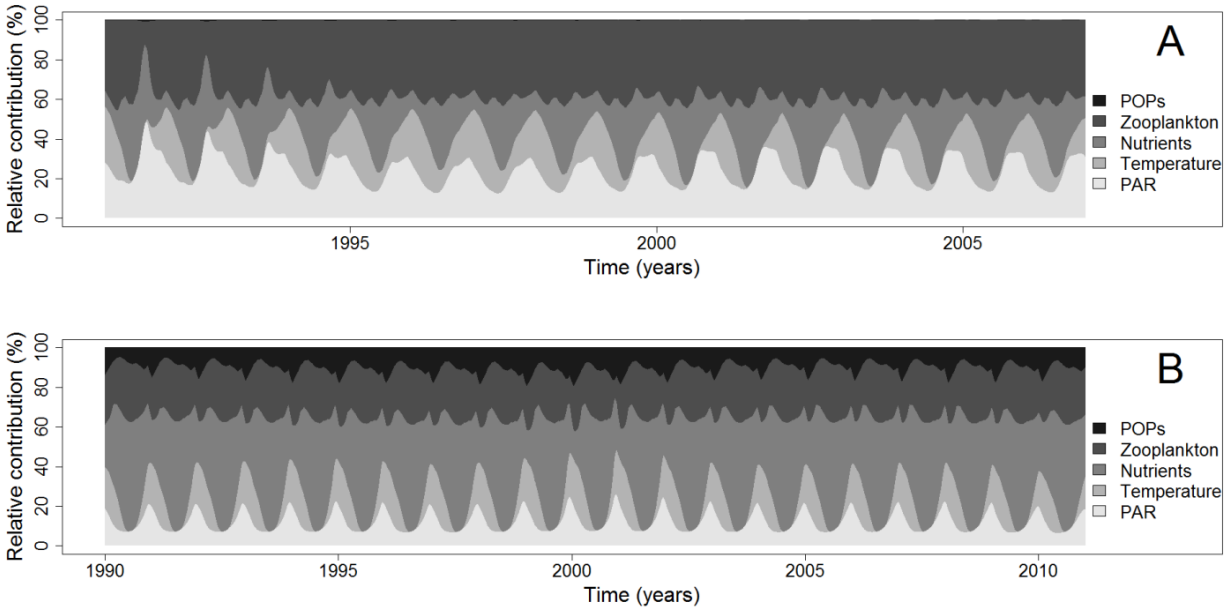


Figure 6.5 *Modeled contribution of photosynthetically active radiation (PAR), water temperature, nutrients, zooplankton grazing and persistent organic pollutants (POPs) to the phytoplankton growth limitation in the Belgian coastal zone (A) and the Kattegat (B). Time trends based on the ten percent best parameterizations of the NPZ model running under the monitored PCB and pesticide concentrations ('1TOX'). The data to produce this figure originate from the limitation terms in the NPZ model. To make this illustration the contribution (in %) of each driver was calculated relative to the total limitation of the phytoplankton growth (the sum of all absolute limitations terms).*

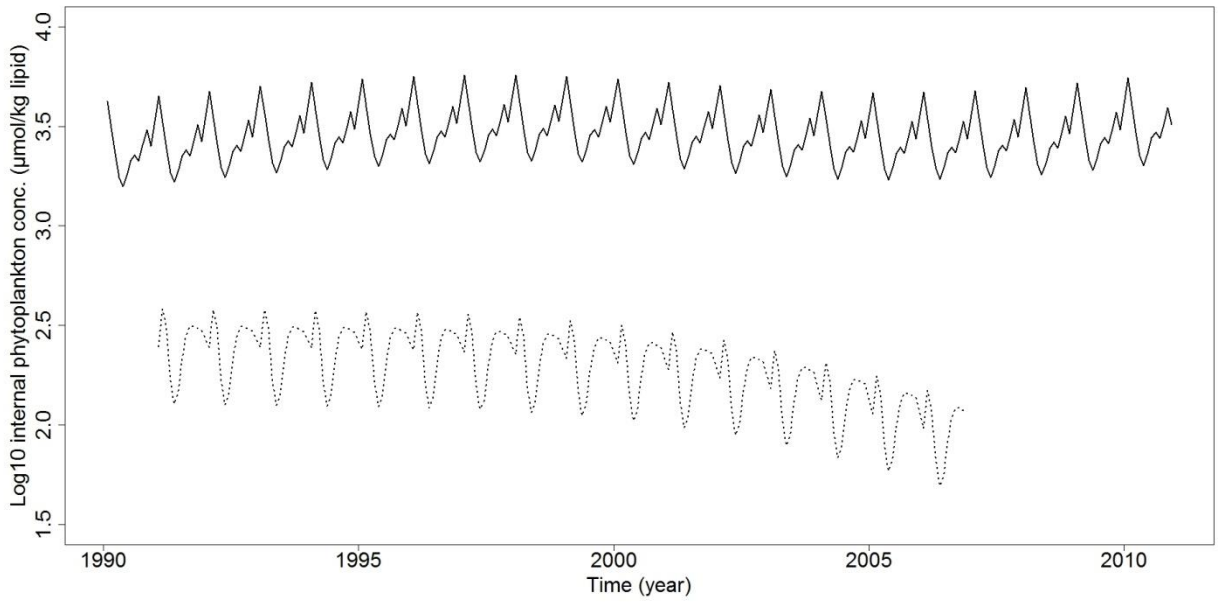


Figure 6.6 *Total concentration of seven polychlorinated biphenyls (PCBs) and four pesticides expressed as internal exposure concentrations in the Belgian coastal zone (dotted line) and the Kattegat (solid line).*

Phytoplankton biomass dynamics simulated by the model configuration including classical drivers and no POPs (0TOX) were accurate as most of the observed chlorophyll a concentrations were within the 95% confidence intervals of the model predictions (Fig. 6.4).

In a next step, we asked whether the unexplained variability of the chlorophyll a concentrations under the 0TOX configuration could be explained by impact of POPs. In this respect, we calculated POP-related contributions to be 1% in the North Sea and 10% in the Kattegat (Fig. 6.5A and 6.5B), suggesting that there are regional differences in the contribution of POPs to the phytoplankton growth limitation. Hence, conclusions from one regional sea cannot be extrapolated to other regional seas due to the presence of point sources (Everaert et al., 2014), differences in secondary POP deposition via latitudinal fractionation (van Waldow et al., 2010) or sea currents (Lohmann et al., 2012) as this may lead to dissimilar regional POP fingerprints and concentrations. Indeed, the POP composition was different between both study regions, i.e. highly hydrophobic POPs were more abundant in the Kattegat study region than in the North Sea study region (Fig. D.9). Also, the internal phytoplankton lipid weight concentrations found in the Kattegat study region (1.57 mmol - 5.74 mmol per kg wet weight) were higher than those found in the North Sea study region (0.05 mmol - 0.38 mmol per kg wet weight) (Fig. 6.6). Based on these internal phytoplankton lipid weight concentrations we expected a POP-induced impact on phytoplankton growth in the Kattegat study region. However, the extension of the NPZ model with a POP-limitation term resulted in better phytoplankton biomass predictions in the North Sea study region (Fig. 6.7A and 6.7B), indicating a POP-induced impact on phytoplankton growth in the North Sea study region and not in the Kattegat study region. The latter was not expected, but might be explained by microevolution processes in the phytoplankton community (Carrera-Martinez et al., 2010). The less exposed community of the North Sea may not be impacted by the current concentrations of POPs (explaining ca. 1% growth inhibition), but an increase in the exposure concentrations of POPs could drastically reduce their growth. On the contrary, the higher exposure of phytoplankton to POPs in the Kattegat may have lead to a selection of tolerant species, and therefore an increase in the POP concentrations could not lead to an increase in the growth inhibition. In this context, Carrera-Martinez et al. (2010) found specific adaptation processes of phytoplankton communities when exposed to organic pollutants. Another reason can be that POPs play a larger role in already stressed communities (e.g. due to nutrient limitations).

From mechanistic point of view it should be noted that the inclusion of an extra explanatory term (here POPs) can also introduce extra uncertainty in the NPZ model. Indeed, prior to the inclusion of the POP-toxicity term some assumptions have been made. A first point of concern is that we only included seven PCBs and four pesticides into the NPZ model, but it is unclear how representative PCBs and pesticides are for the combined effect of all POPs. Time trend studies indicate decreasing PCB and pesticide concentrations in the marine environment (Venier et al., 2012; Gioia et al., 2008; Everaert et al., 2014; Roose et al., 1998), but environmental concentrations of emerging hydrophobic compounds such as perfluorooctane sulfonic acid and perfluorooctanoic acid (Holmström et al., 2005), pharmaceuticals and antibiotics (Gaw et al., 2014) are stable or even increasing. Although those emerging compounds also partition towards and impact on biota (de Wit et al., 2010), the effects and trends of these compounds are not directly included in the NPZ model. The main reason why those substances were not included in the NPZ model is the limited amount of long-term monitoring data in the marine environment (Law et al., 2010). A second point of concern,

which is to a certain extend related to the first one, is that the knowledge on the interactions between multiple drivers (not only basic drivers, but also chemical pollutants) on marine primary production is incomplete (Echeveste et al., 2010). Based on the funnel hypothesis it was assumed in the present paper that the marine POPs (present at low concentrations) act in an additive way (Warne and Hawker, 1995). However, because of the heterogeneity of substances and their various modes of toxic action (Escher et al., 2011), interactions between organic chemicals in a mixture may also result in a weaker (antagonistic) or stronger (synergistic) combined effect than suggested by concentration addition. Thus, additive, synergistic and antagonistic effects may be taken into account in future POP toxicity assessments (Altenburger et al., 2003).

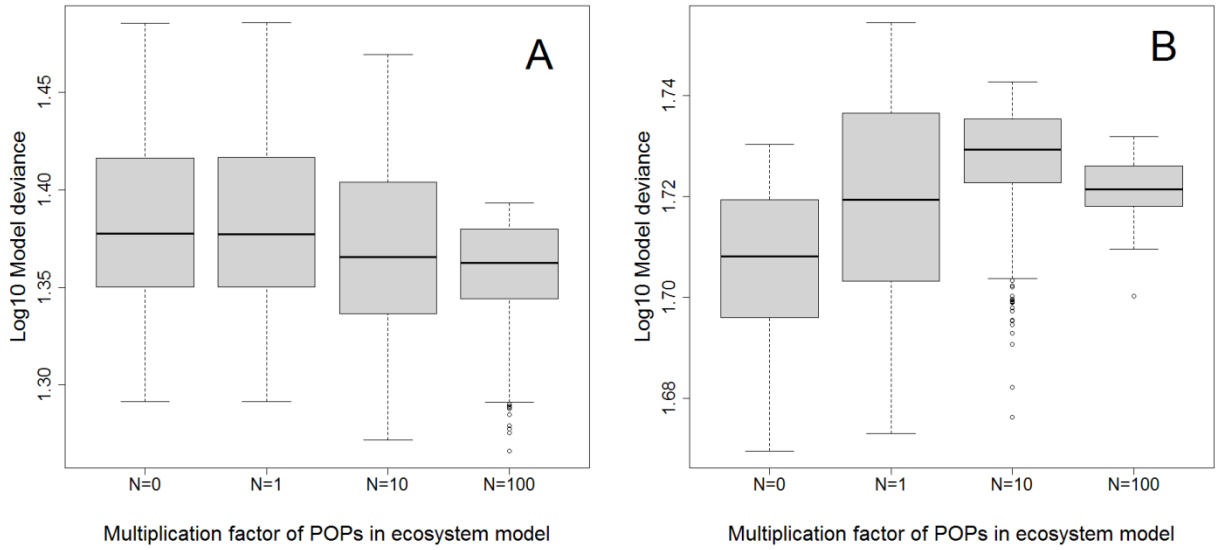


Figure 6.7 *Box and whisker plots of the model deviations for four nutrient-phytoplankton-zooplankton (NPZ) model configurations assuming: no persistent organic pollutants (POPs) present (N = 0;'0TOX'), monitored POP concentrations (N = 1; '1TOX'), a ten-fold of the monitored POP concentrations (N = 10; '10TOX') and a hundred-fold of the monitored POP concentrations (N = 100; '100TOX'). Based on the ten percent best parameterizations in the Belgian coastal zone (A) and in the Kattegat (B).*

One of the open questions is whether the part of the phytoplankton biomass dynamics not explained by classical drivers in the NPZ model (light, nutrients, temperature and zooplankton grazing) can be uniquely related to the presence of POPs. Also iron availability (Geider and la roche, 1994), global warming (Dalpadado et al., 2014) and sinking and cell lysis (Agusti et al., 1998) may have (in)direct effects on marine primary production. Therefore, to better distinguish between natural variability in phytoplankton growth and anthropogenic driven shifts it is recommended to also include these drivers additional to POPs in the NPZ model. However, due to limited spatiotemporal data coverage other confounding factors have not been included in the present paper. Furthermore, the NPZ model can also be extended with a factor describing the toxicity of POPs to zooplankton. However, the validity of the presented approach for assessing the ecotoxicological effect of POPs to higher trophic levels such as zooplankton and commercially important (fish) species is a topic for future research.

6.4 Conclusion

In the present research, we present a model-based approach to disentangle the relative importance of light and nutrient availability, temperature, zooplankton grazing and persistent organic pollutants on the phytoplankton growth. The integrative ecosystem modelling approach presented in this study may help to separate anthropogenic effects from natural variability. Based on almost two (North Sea study region) and three (Kattegat study region) decades of data the present paper revealed that seven PCBs and four pesticides did not hamper phytoplankton growth at the monitored concentrations. Under the elevated POP concentrations, POP-impact was region dependent but not a major driver (Fig. 6.4 and 6.5).

7

General conclusions

7.1 Introduction

The current knowledge about ecotoxicological effects of organic chemicals on marine ecosystems is limited to few substances and few species. Hence, the overall impact of chemical substances on marine ecosystems is largely unknown. To partly address this limitation, here we assessed - using phytoplankton - how marine ecosystems respond to the presence of organic chemicals. By analyzing existing data and performing laboratory experiments, ecotoxicological effects on marine organisms and ecosystem functions are quantified. The relative impact of organic chemicals to phytoplankton growth dynamics (in comparison to natural drivers such as nutrients, temperature and light) is estimated.

This chapter reviews the results from this dissertation, summarizes the conclusions and offers suggestions for further research. The main conclusions are summarized and related to the original research questions. The structure presented in paragraph 1.6 is followed and illustrated in Figure 7.1. For each chapter, main conclusions are shown in Figure 7.2. Suggestions for further research are provided and links between different chapters are highlighted. Each section starts with the research question addressed and compiles the conclusions drawn.

7.2 Spatiotemporal trends of polychlorinated biphenyls along the Belgian coast and in the Western Scheldt estuary

→ Chapter II: How did PCB concentrations in sediments change along the Belgian coast and in the Western Scheldt estuary?

→ Chapter II: Are the spatiotemporal trends of PCB concentrations along the Belgian coast and in the Western Scheldt estuary driven by primary emissions or secondary emissions?

The spatiotemporal trends that were obtained confirmed the research hypotheses: (1) decreasing interannual PCB concentrations were found in open waters (Fig.7.2B); (2) less decreasing or even stable long-term PCB concentrations were found in the urbanized and industrialized estuary (Fig.7.2C); and (3) concentrations of PCBs in sediments of estuary exceeded open sea conditions (Fig.7.2D). Model-based predictions using spatiotemporal information (sampling year and sampling location), the name of the congener and a periodicity factor explained 69% of the variability in the observations. When applying the model to independent data, predicted sediment concentrations compared well to the observed concentrations (Fig. 7.2A). It was found that secondary emissions from historically polluted environmental compartments sources have more impact on PCB concentrations than emissions from primary sources. These results demonstrate that international efforts to cut down emissions of PCBs have been effective to reduce concentrations in open water ecosystems like the BCZ but had little effect in the urbanized and industrialized area of the Scheldt estuary.

→ Suggestions and recommendations for further research

International monitoring programs and individual research projects collected a large amount of physical-chemical and biological data in marine ecosystems. By archiving

these data, the ICES data repository, for example, contains more than 286,000,000 oceanographic data and 11,000,000 contaminant and biological effects data collected between 1862 and 2014 (see www.ices.dk). These data can be used to perform meta-analyses (e.g. Irwin and Finkel, 2008) and can be consulted to answer scientific questions different from the initial purpose for which the data were collected (e.g. Everaert et al., 2014). Data should be analyzed in a systematic and transparent manner for which existing protocols (e.g. Zuur et al., 2010) can be used. By analyzing existing data, marine ecosystems can be better understood, knowledge gaps can be identified and used to define future marine research topics. Overall, existing data can improve the knowledge about marine ecosystems, so it is needed to further collect, maintain and integrate scattered marine biological, oceanographic and contaminants data.

7.3 Spatiotemporal trends of polychlorinated biphenyls in the North Sea and Celtic Sea

→ Chapter III: How did concentrations of organic contaminants in sediment and biota change in the North Sea and the Celtic Sea between 1979 and 2012?

Decreasing interannual PCB concentrations were found in North Sea sediments (Fig. 7.2E) and mussels (Fig. 7.2F). However, PCB concentrations in sediments showed, less than PCB levels in mussels, decreasing interannual trends. In the Celtic Sea, decreasing interannual PCB concentrations in mussels and stable interannual PCB concentrations in sediments were inferred. The latter probably related to the fact that sediment samples in the Celtic Sea were predominantly sampled in coastal waters and estuaries (Fig. 3.5). Interannual changes in PCB concentrations were separated from seasonal variability. By doing so, superimposed to the generally decreasing interannual trends, seasonally variable PCB concentrations were observed in line with findings of Galban-Malagon et al. (2012) (Fig.7.2G and 7.2H). Consistent with Konat and Kowaleska et al. (2007), PCB concentrations in sediments decreased when samples were taken further away from the coastline (Fig.7.2I),

→ Suggestions and recommendations for further research

In the present study spatiotemporal trends of PCBs were investigated. Focus was on PCBs, which are priority chemicals with legislatively restricted primary emissions. Interesting conclusions were drawn, but the question is how relevant the spatiotemporal trends of PCBs are for other organic chemicals that are not frequently monitored and assessed. Therefore, it is needed to select new and emerging chemicals that end up in the marine environment, but for which the concentrations and potential environmental risks (see further) are currently poorly studied. To do so, a literature review can be performed and in a next step, these emerging chemicals can be incorporated in existing monitoring and assessment programmes.

7.4 Coupling between polychlorinated biphenyl concentrations in three environmental compartments

→ Chapter III: Are concentration changes of organic contaminants interrelated between different environmental compartments and linked to the seasonal phytoplankton cycle?

Separating the interannual changes from seasonal variability (based on additive modelling, chapter III) confirmed the tight coupling of PCB concentrations between environmental compartments (Fig. 7.2K). In agreement with the theoretical description of Dachs et al. (1999) and field observations by Berrojalbiz et al (2011) and Galban-Malagon et al. (2012), we found strong and significant correlations between seasonal changes of chlorophyll a concentrations and seasonal changes of PCB concentrations (Fig.7.2K). The phytoplankton blooming events in spring and autumn correspond to the annual maxima of PCB concentrations in the sediment (Fig.7.2G). Minimum PCB concentrations in mussel tissue are found in summer and related to the low concentrations of freely dissolved PCBs in this period and are an indirect effect of the phytoplankton blooming event (Fig.7.2H).
The novelty of the study lies in the fact that the working of the biological pump was, to our knowledge, never assessed based on field data collected at a regional scale in multiple decades. In 2012, Nizzetto et al. stated that from theoretical point of view, the biological pump is rather well understood, but that experimental assessments and field data have never been used as evidence of this phenomenon. Until now, often data were collected in different environmental compartments in one growing season close to the navigational route of research vessels (Berrojalbiz et al., 2011; Galban-Malagon et al., 2012). These data yield interesting quantifications of the fluxes of organic contaminants between different environmental compartments. In the present research, although the data were collected in the very dynamic and heterogeneous environment of the Celtic and North Sea, we were able to derive, not only an interannual trend (Fig. 7.2E and 7.2F), but also a seasonal fingerprint of the biological pump in the PCB concentrations in the water (Fig. 3.9), sediment (Fig. 7.2G) and biota (Fig. 7.2H). Overall, biogeochemical processes are major drivers in the fate of organic contaminants and changes in these processes may have serious impacts on the chemical pollution status of the marine environment.

→ Suggestions and recommendations for further research

To study the role of biogeochemical processes (e.g. the biological pump) on the fate of POPs focus has been mainly on priority substances such as PCBs and PAHs (e.g. Berrojalbiz et al., 2011; Galban-Malagon et al., 2012). Emissions of these priority substances are restricted to reduce levels of PCBs to zero and levels of PAHs to natural background concentrations (OSPAR, 2010). For emerging contaminants (e.g. pharmaceuticals, antibiotics, personal care products etc.) emissions are not (yet) restricted, making the impact of biogeochemical processes on the fate of these hydrophobic emerging contaminants not well understood. Therefore, the role of biogeochemical processes on the fate of emerging organic chemicals requires further investigation.

→ Suggestions and recommendations for further research

The influence of the biological pump is not only present in open waters of the Atlantic and Arctic oceans (Jaward et al., 2004; Galban-Malagon et al., 2012) and the Mediterranean Sea (Berrojalbiz et al., 2011), but also in the very dynamic and heterogeneous North Sea and the Celtic Sea which have a long history of chemical pollution (Chapter III). In this context, a next step is to quantify the fluxes of organic chemicals between different environmental compartments (to reach equilibrium, cfr. 1.3.1). These fluxes have been quantified in the Atlantic and Arctic ocean and the Mediterranean Sea, but so far no knowledge is available for others seas and oceans.

→ Suggestions and recommendations for further research

Fluxes of POPs between different environmental compartments have been often quantified (e.g. Sundqvist et al., 2004; Meijer et al., 2009; Galban-Malagon et al., 2013). However, whether and, if present, how these fluxes have changed in the last decades is poorly studied. In this context, potential changes in the fluxes of POPs between the environmental compartments can be related with human-induced impacts on the marine environment and provide (additional) evidence for the human impact on marine biogeochemical processes.

7.5 Risk quotient of polychlorinated biphenyls changes on a spatiotemporal scale

→ Chapter IV: How did the potential risk of organic contaminants change in the North Sea and the Celtic Sea between 1979 and 2012 according to the environmental quality thresholds?

Risk quotients based on PCB concentrations in open sea sediments (> 200km offshore) remained below the value of 1 between 1979 and 2012, indicating no potential ecotoxicological risk. In coastal sediments however, PCBs posed potential adverse effects between 1979 and 2012 (Fig. 7.2L). Risks quotients based on PCB concentrations in mussels in the 1980s were two orders of magnitude higher than the corresponding quality threshold. To date, following the decreasing PCB concentrations in biota (Chapter III), PCB concentrations in mussels only pose a potential risk along the North Sea coasts (Fig.7.2M). An important extra conclusion is that not only interannual changes in PCB concentrations should be taken into account when assessing the risk of PCBs. Also the spatiotemporal variability due to seasonal changes (Fig. 7.2G and 7.2H) and spatial changes (Fig. 7.2I and 7.2J) should be considered. Indeed, the impact of the biological pump on the PCB concentrations (Chapter III) is translated into the risk quotients obtained (Fig.7.2L and 7.2M).

→ Suggestions and recommendations for further research

Quality threshold are mainly used as early warning signals (Langston et al., 2012), i.e. when the risk quotient exceeds the value of 1, additional biological investigations should be initiated. As such, there is no causal relationship between exceeding a quality threshold and the observed ecotoxicological effects. Indeed, quality thresholds

are associated with large uncertainty levels (Wu et al., 2008). Environmental assessment criteria (EACs), for example, have a range of one order of magnitude (OSPAR, 1998). There is urgent need for additional ecotoxicological data to minimize the uncertainty when using EACs and to allow a better linkage between risk quotients and –potential- adverse effects.

7.6 Mixture effects of organic chemicals on a marine diatom

→ Chapter V: Does a realistic mixture of organic contaminants alter the growth dynamics of a marine diatom under laboratory conditions?

Exposure of *Phaeodactylum tricornutum* to a realistic mixture of organic pollutants present in Belgian marine waters did not change its specific growth rate (Fig. 7.2N). Based on laboratory experiments with passive samplers, a realistic mixture of organic chemicals close to ambient concentrations did not exert direct ecotoxicological effects to a marine diatom. Overall, based on an algal growth inhibition test with *P. tricornutum*, being an intermediate pollution sensitive marine diatom, it is suggested that the natural occurring concentrations of organic chemicals present along the Belgian coast are not likely to affect the growth of marine diatoms.

→ Suggestions and recommendations for further research

Passive sampling and dosing is useful to screen the ecotoxicological potential of realistic environmental mixtures (Chapter V). In case that an ecotoxicological effect is detected, advanced analytical methods are needed to identify causative chemicals. In chapter V, we analyzed the passive sampler extracts for PCBs and PAHs. However, passive samplers accumulate all organic chemicals for which they have affinity, i.e. not only PCBs and PAHs are present on the passive samplers (Rusina et al., 2007; Claessens, 2013). To identify the causative chemical(s), high-resolution full scan analysis is needed. Instruments such as orbitrap mass spectrometer may help to identify the chemicals responsible for the ecotoxicological effects observed (Wille et al., 2011). Furthermore, attention should be paid to the passive sampler material that is used to perform the monitoring and assessment (Vrana et al., 2005). Each passive sampler material has its own affinity for target organic chemicals. In this context, the use of multiple materials that differ in their affinity for the target organic chemicals is recommended to cover the entire range of organic chemicals (ranging from very hydrophobic to hydrophilic compounds; Claessens, 2013).

→ Suggestions and recommendations for further research

Although the present research yields interesting conclusions for one diatom (i.e. *P. tricornutum*), it is suggested to assess the validity of the results in a mesocosm experiment (including multiple species and different trophic levels) and/or under field conditions. Based on the present research, no ecotoxicological conclusions can be drawn for the effects of POPs to other plankton groups or higher trophic levels because *P. tricornutum* only represents the first trophic level in the marine food-web.

7.7 Relative contribution of organic chemicals to phytoplankton growth

→ Chapter V and VI: What is the relative contribution of organic chemicals, nutrients, light availability and temperature to the growth dynamics of marine phytoplankton?

Natural drivers of phytoplankton growth such as nutrients, temperature and light availability, explaining about 85% of the observed variability, are much more important drivers than naturally occurring mixtures of organic pollutants present in Belgian marine waters (Chapter V). Indeed, exposure to a natural mixture of hydrophobic contaminants did not change the specific growth rate of *Phaeodactylum tricornutum* (Fig. 7.2N). The main effect of organic pollutants to the growth of the marine diatom was limited to about 1% and not significant (Fig. 7.2O).
In the model-based approach (Chapter VI), POP-induced growth limitation of marine phytoplankton was small compared to the limitation caused by the natural drivers (Fig.7.2P). As in chapter V, we estimated the relative contribution of POPs to the phytoplankton growth dynamics to be about 1% along the Belgian coast (Fig.7.2Q). In the Kattegat however, this was about 10% indicating regional differences of POP-induced effects.

→ Suggestions and recommendations for further research

Biodiversity protects ecosystems from changes in their functionality and makes them more resilient to changing environmental conditions (Naeem et al., 2012; Cardinale et al., 2012). According to the functional redundancy hypothesis, high biodiversity levels guarantee that if a species disappears, another species with the same functional role will maintain the ecosystem's functionality (Loreau, 2004). As such, possible ecotoxicological effects on individual species may remain unnoticed when only looking at the "bulk" primary production. To further unravel potential ecotoxicological effects, focus should be on descriptors underlying the "bulk" primary production (e.g. species occurrences, abundances, genomics, etc.). To do so, field surveys in hot spot areas, mesocosm experiments and modelling exercises can help to identify and quantify ecotoxicological effects of natural mixtures of chemicals.

→ Suggestions and recommendations for further research

Humans alter marine ecosystems in multiple ways. Oceans are becoming warmer and more acidic as a consequence of anthropogenic CO_2 emissions (Levitus et al., 2000; Doney et al., 2009). A growing body of literature illustrates the negative impacts of these factors (warming and acidification) on marine ecosystem resilience. However, the combined effects of natural stressors and stress due to chemical pollution on ecological communities remain largely unknown (Holmstrup et al., 2010). The increasing demand for understanding the response of marine ecosystems to chemical stress and climate change highlights the need to develop and improve integrated experimental designs and descriptive and predictive ecological models. There is a need for novel strategies to assess potential adverse biological effects of mixtures of multiple stressors at different levels of biological organization (Holmstrup et al., 2010).

→ Suggestions and recommendations for further research

In this study, we focused on the first trophic level of the marine food web. However, POPs accumulate in the food chain and highest POP concentrations are typically found at the highest trophic levels (e.g. predatory bird, fish and mammals) where ecotoxicological effects are expressed (e.g. Barron et al., 2003; Letcher et al., 2010). Although effects at these trophic levels are most visible, it is likely that also lower trophic levels are affected. As such, the question is from which point in the trophic food web the exposure to POPs and the accumulated burden turns out to be problematic. To do so, bioaccumulation models such as the one proposed by Hendriks et al. (2001) can be used.

7.8 Overall contributions of this dissertation to science

This dissertation evaluated the potential risk of organic micropollutants in the marine environment. By integration of existing monitoring data, experimental data and advanced statistical analyses, it is shown that the spatiotemporal variability of PCB concentrations should be considered in future environmental risk assessments and that ambient concentrations of the organic chemicals present are not major drivers of phytoplankton growth. Concentrations of PCBs and pesticides have generally decreased over time in open waters. In coastal waters and in estuaries, however, concentrations remained relatively unchanged. These results demonstrate that international efforts to decrease emissions of PCBs have been effective to reduce concentrations in open water ecosystems, but had little effect in urbanized and industrialized estuaries. The latter is reflected in the relatively high risk quotients (>1) close to the coast. Based on the outcome of the traditional risk assessment, two extra analyses, one model-based approach and one laboratory-based experiment, were performed. Despite the fact that the risk assessment identified a potential ecotoxicological effect of organic chemicals, neither the lab-based study, nor the model-based study indicated that organic chemicals have a major ecotoxicological effect on marine phytoplankton. By contrast, it was demonstrated that phytoplankton (biological pump) is an important driver in the fate of POPs. The latter is a novel result since the effectiveness of the biological pump was never assessed before based on field data collected at the scale of a regional sea in multiple decades. Overall, natural factors such as nutrients, temperature, light availability and zooplankton grazing are much more important drivers of the marine phytoplankton growth than organic chemicals. In future research, the validity of these conclusions should be further assessed for other substances, other species and higher trophic levels.

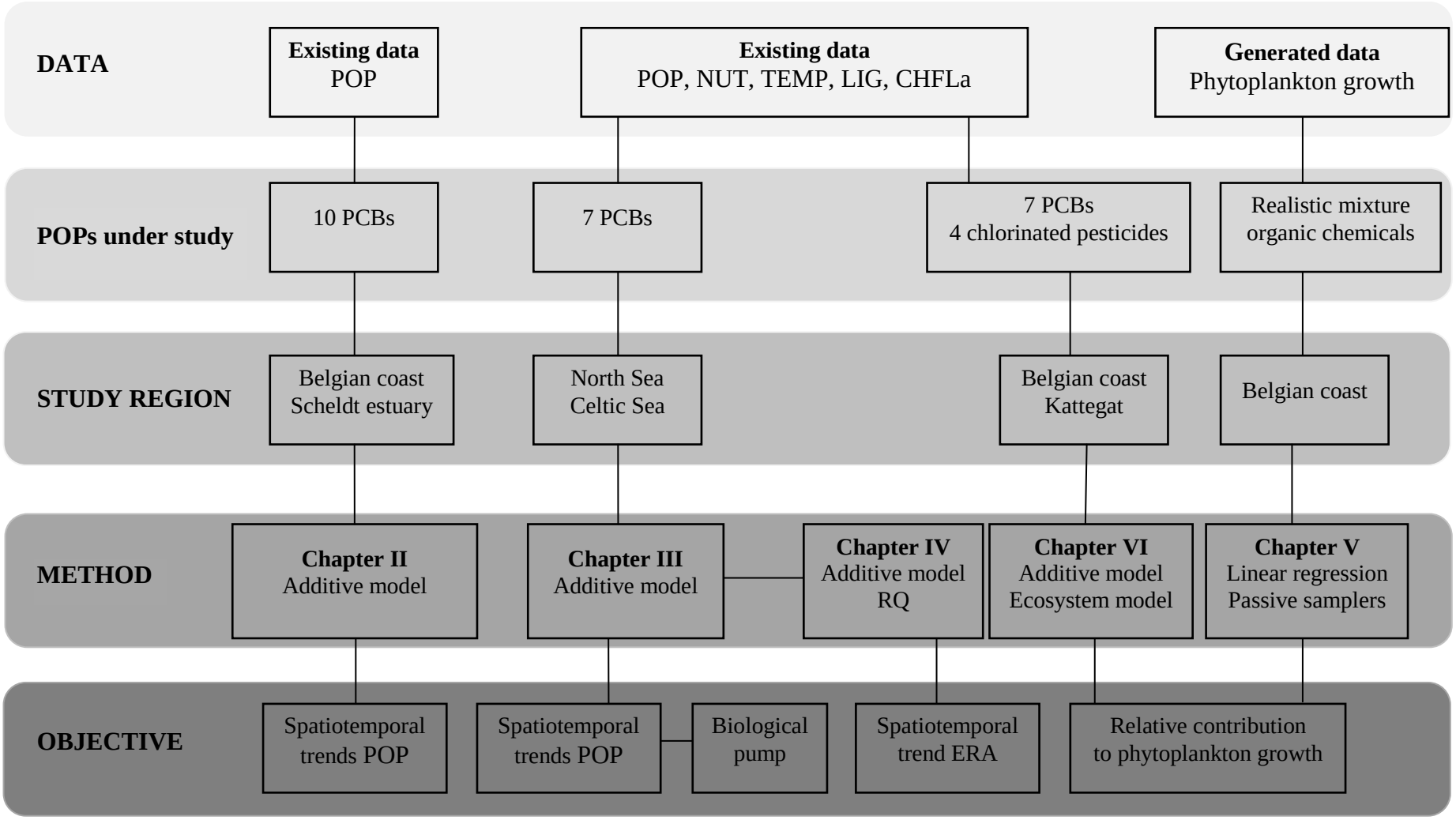
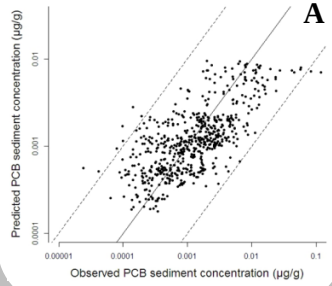


Figure 7.1 *Schematic overview of the data used, the persistent organic pollutants studied, the study region, the methodology and the research objectives for each chapter included in this dissertation.*

Chapter II

Model fit

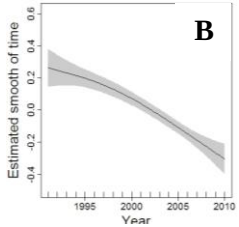
A



Interannual time trend

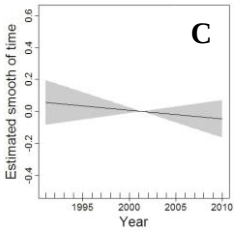
Open sea

B



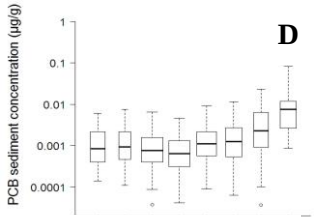
Scheldt estuary

C



Spatial trend

D



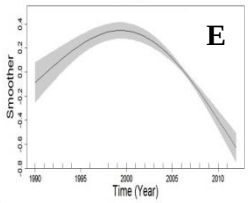
Open sea → estuary

Chapter III & IV

Interannual time trend

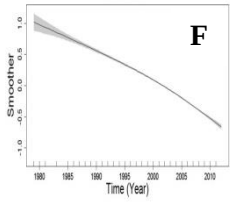
Sediment

E



Mussel

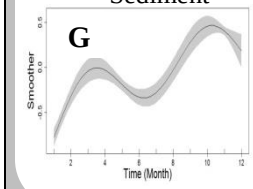
F



Seasonal time trend

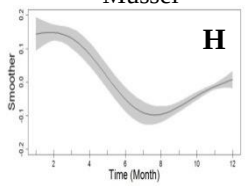
Sediment

G



Mussel

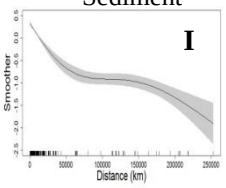
H



Spatial trend

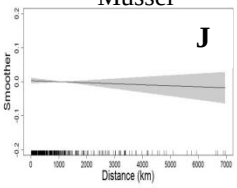
Sediment

I



Mussel

J



Biological pump

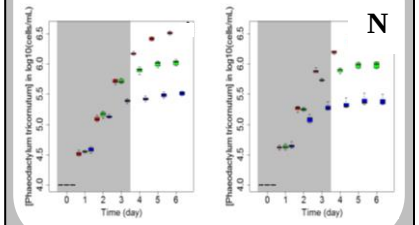
K

	$[PCB]_{sediment}$	$[PCB]_{mussel}$	$[CHFLa]_{water}$
North Sea			
$[PCB]_{sediment}$	-		
$[PCB]_{mussel}$	**-0.78** (0.003)	-	
$[CHFLa]_{water}$	**0.48** (0.011)	-0.34 (0.270)	-
$[OC]_{sediment}$	**0.77** (0.003)	-0.50 (0.095)	**0.71** (0.010)
Celtic Sea			
$[PCB]_{sediment}$	-		
$[PCB]_{mussel}$	**-0.76** (0.004)	-	
$[CHFLa]_{water}$	**0.61** (0.037)	-0.33 (0.290)	-
$[OC]_{sediment}$	**0.74** (0.006)	-0.52 (0.085)	**0.71** (0.010)

Chapter V

Realistic mixture

N



Laboratory-based contribution

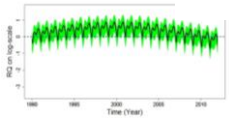
O

Model	Model configuration	Variability explained
1	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM + (DAY + DAY²) * LIG + (DAY + DAY²) * ORC	88.1
2	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM + (DAY + DAY²) * LIG	87.0
3	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM	85.2
4	(DAY + DAY²) * NUT	66.6
5	DAY + DAY²	12.8
6	DAY	10.7
7	(DAY + DAY²) * NUT + (DAY + DAY²) * TEM + (DAY + DAY²) * LIG + ORC:NUT + ORC:TEM + ORC:LIG	90.8

Time trend risk quotient

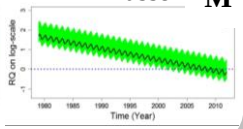
Sediment

L



Mussel

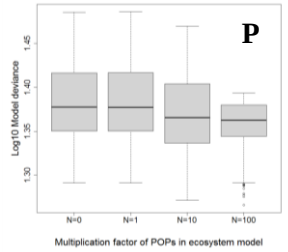
M



Chapter VI

Ecosystem-based contribution

P



Q

	Belgian coast	Kattegat
Nutrients	10 – 40%	10 – 20%
Temperature	0 – 30%	0 – 30%
PAR	20 – 50%	10 – 20%
Zooplankton	20 – 50%	20 – 30%
POPs	0 – 2%	10 – 20%

Figure 7.2 *Overview of the most important results of the five main chapter of this dissertation. Letters A-Q are used to refer to the figures. Enlarged copies of the figures and tables are found in the main text of the corresponding chapters.*

A

Supportive information for chapter 2

Figures

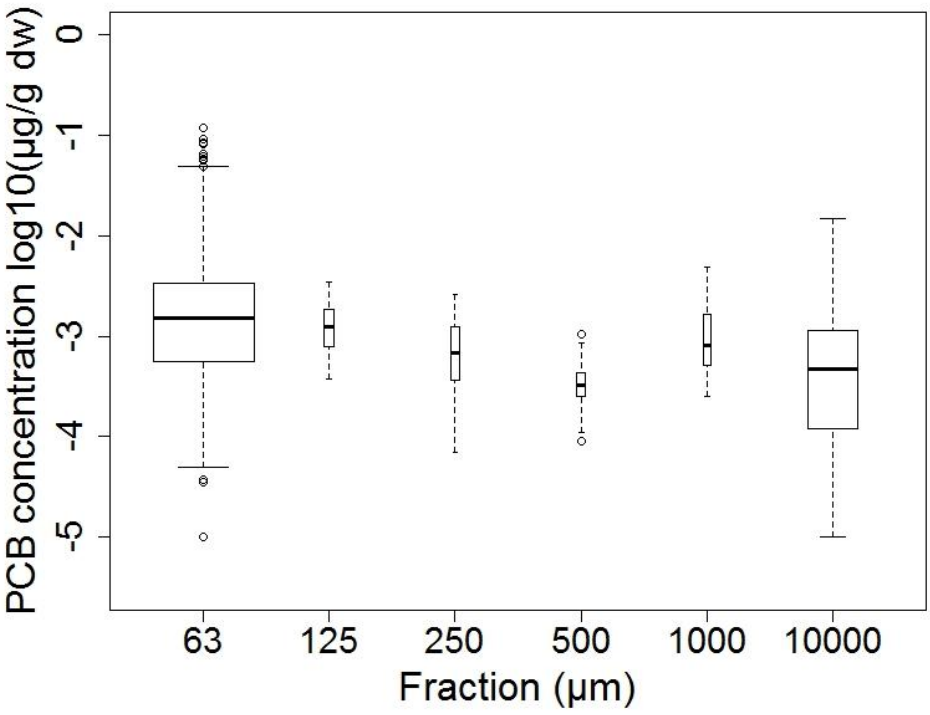


Figure A.1 *Data available for each fraction (63 = fraction smaller than 63 µm; 125 = fraction smaller than 125 µm; 250 = fraction smaller than 250 µm; 500 = fraction smaller than 500 µm; 1000 = fraction smaller than 1000 µm; 10000 = fraction smaller than 10000 µm).*

In total, 376 values were discarded because it was indicated in the BMDC data repository that the PCB concentrations of these samples were below the detection limit. There were three main reasons to discard these data. First, when performing a parallel modelling exercise including these 376 concentrations, noise was added in the statistical analyses. The latter is reflected in the biased QQ plot and skewed histogram (Fig. A.2). Also, when plotting the residuals versus fitted values and the responses versus fitted values the detection limit values are clearly identifiable (Fig. A.2 – A.3). Moreover, when including the 376 concentrations below the detection limit, higher AIC values (model 1: 1583.4, model 2: 2042.3, model 3: 1965.3) and lower R^2 values (model 1: 0.54, model 2: 0.34, model 3: 0.33) were found compared to the analyses reported in the main text (Table 2.1). Second, taking half of the concentrations and include them in the analysis was no appropriate solution. Although within the entire timeframe (1991-2010) concentrations of PCB were quantified using the same gas chromatography - electron capture detector (GC-ECD) in the same laboratory, different levels of detection limits were reported (i.e. 10^{-4} µg.g^{-1}, 10^{-5} µg.g^{-1}, $1.6*10^{-8}$ µg.g^{-1}, $1.6*10^{-5}$ µg.g^{-1}, etc). Hence, the reported detection limits could not be trusted. Finally, only 376 data were discarded which is less than 10% of all data (in total 4297) considered.

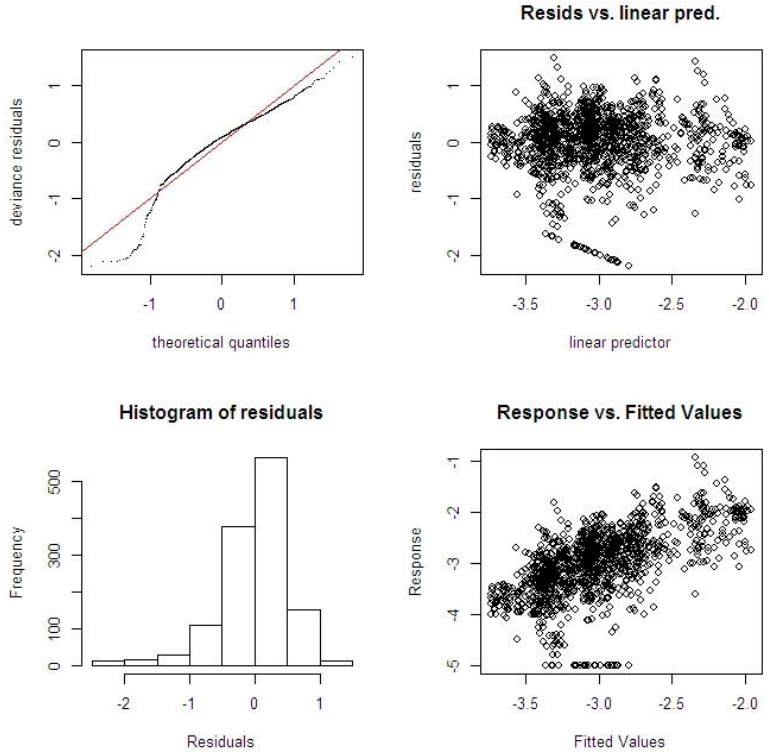


Figure A.2 *Validation tools for the generalized additive mixed model that contains a smoother for time, periodicity and log K_{oc} and two nominal variables being the sampling zone PCB cluster. The QQ plot and the histogram are used to assess normality, and the residuals versus fitted values homogeneity. The response versus fitted values should ideally show a straight line. Detection limit values are included in these analyses.*

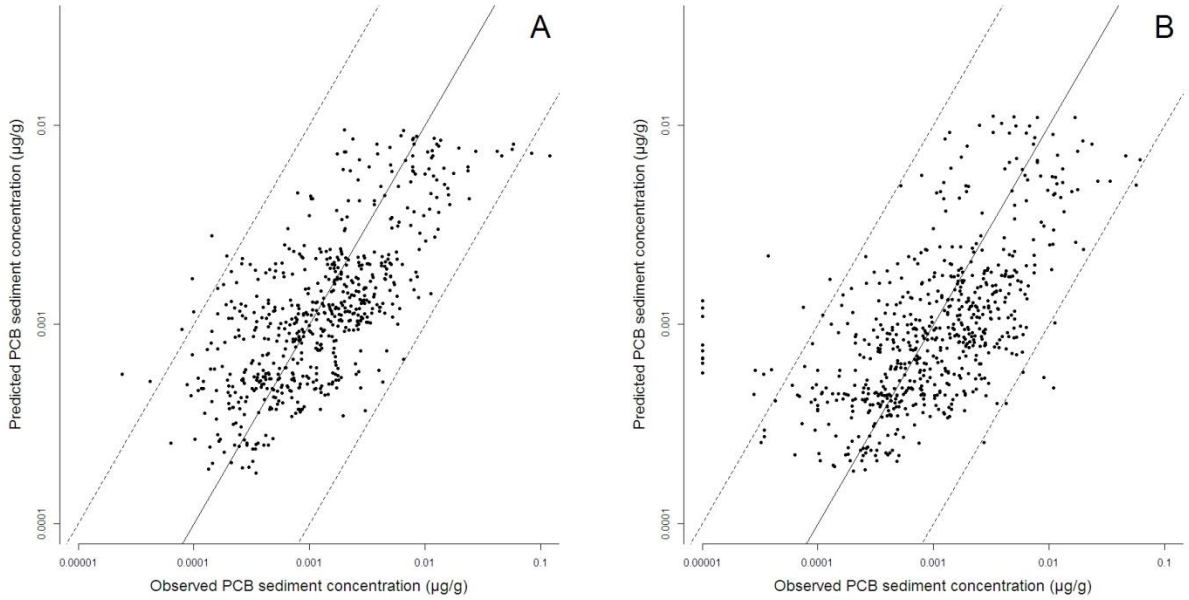


Figure A.3 *Application of model 3 on the independent data set (n=614). The estimated PCB sediment concentrations are compared with the observed concentrations. The solid line represents the first bisector and the dotted lines represent one order of magnitude deviance. Model 3 was based on a dataset discarding the detection limit values (panel A, see also Fig. 2.4) and including the detection limit values (panel B).*

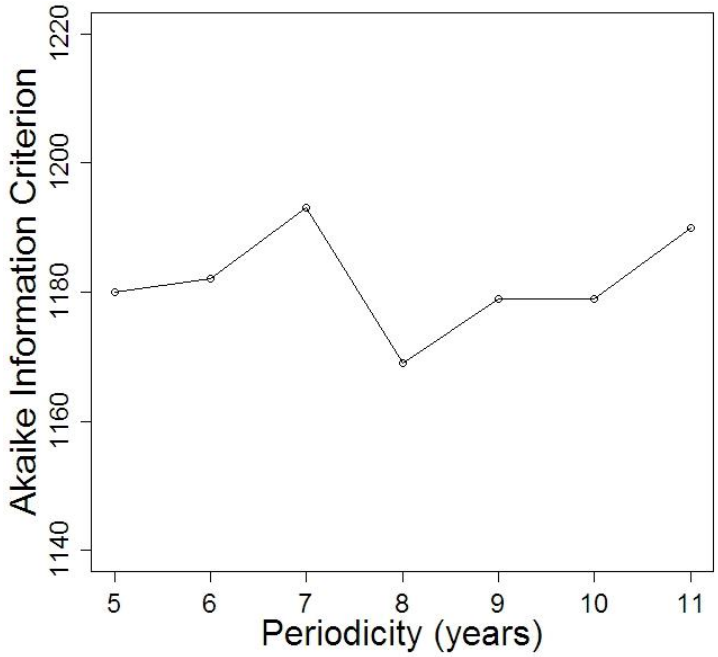


Figure A.4 *Akaike Information Criterion (AIC) of the generalized additive mixed model (model 3) as a function of the periodicity term.*

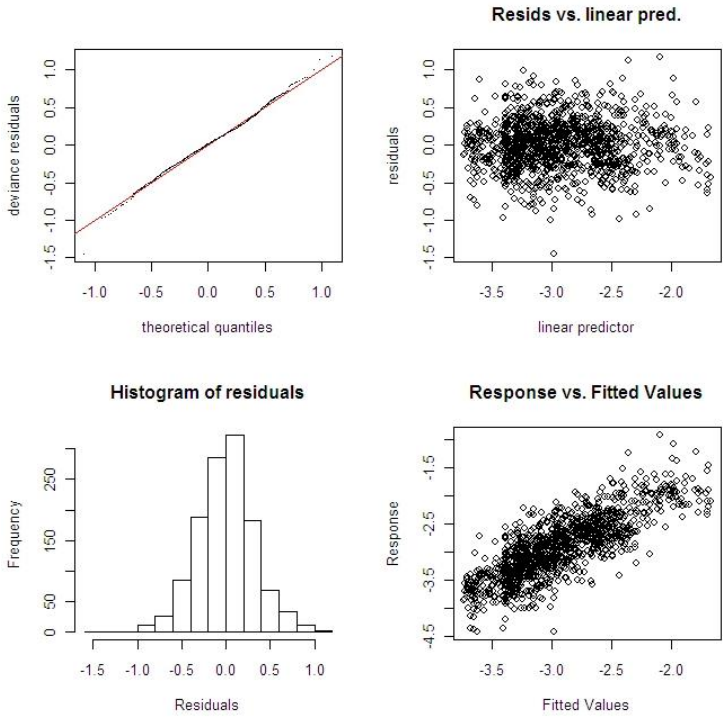


Figure A.5 *Validation tools for the generalized additive mixed model that contains a smoother for time, periodicity and log K_{oc} and two nominal variables being the sampling zone PCB cluster. The QQ plot and the histogram are used to assess normality, and the residuals versus fitted values homogeneity. The response versus fitted values should ideally show a straight line. Detection limit values are discarded in these analyses.*

Tables

Table A.1 *PCB data available in different fraction in the Belgian Coastal Zone and the Western Scheldt estuary. Fractions indicate ….*

PCB congener	< 63 µm	< 125 µm	< 250 µm	< 500 µm	< 1000 µm	< 10,000 µm
CB28	326	4	4	4	4	112
CB31	322	NA	NA	NA	NA	NA
CB52	326	4	4	4	4	112
CB101	326	4	4	4	4	112
CB105	322	NA	NA	NA	NA	NA
CB118	326	4	4	4	4	112
CB138	325	4	4	4	4	112
CB153	326	4	4	4	4	112
CB156	322	NA	NA	NA	NA	NA
CB180	326	4	4	4	4	111

Table A.2 *Outliers identified based on the Cleveland dotplots*

Sampling location	Sampling date	Sampling campaign	Substance(s)
ZVL_DVZ	21/09/2010	BE2010/25A	PCB 105 and PCB 52
B08	10/03/1997	BE1997/06A	PCB 138

Table A.3 *Summary of the data used for fitting the models per PCB per zone: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

	Log	Sampling year		Zones								Total
PCB	**K_{oc}**	**Min.**	**Max.**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**data**
28	4.178	1991	2010	13	8	26	24	23	11	11	11	127
31	4.217	1991	2010	7	6	22	22	21	9	10	15	112
52	4.438	1991	2010	9	7	22	24	24	15	9	13	123
101	4.831	1991	2010	9	10	27	24	22	14	10	12	128
105	4.93	1992	2010	12	5	23	16	17	13	14	14	114
118	5.008	1991	2010	15	6	28	23	18	11	11	15	127
138	5.185	1991	2010	11	9	25	22	20	13	10	17	127
156	5.273	1991	2010	10	5	17	21	14	14	13	12	106
153	5.356	1991	2009	12	10	25	22	18	15	13	15	130
180	5.644	1991	2010	13	6	24	24	19	14	14	11	125

Table A.4 *Summary of the data used for model validation per PCB per zone: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

	Log	Sampling Year		Zones								Total
PCB	K_{oc}	Min.	Max.	A	B	C	D	E	F	G	H	data
28	4.178	1991	2010	6	3	10	13	8	9	6	9	64
31	4.217	1991	2010	7	4	9	9	7	10	6	5	57
52	4.438	1991	2010	6	3	12	13	7	5	9	7	62
101	4.831	1991	2010	10	1	9	13	9	7	8	8	65
105	4.93	1991	2010	4	2	9	15	10	7	4	6	57
118	5.008	1991	2010	4	5	8	14	13	9	6	5	64
138	5.185	1992	2010	7	2	10	15	11	8	8	3	64
156	5.273	1991	2010	4	1	10	8	12	5	5	8	53
153	5.356	1991	2010	7	1	11	17	13	6	5	5	65
180	5.644	1991	2009	5	4	11	12	11	7	4	9	63

Table A.5 *Summary of the data used for model training per zone over time: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

	Sampling year					
Zone	Minimum	First quartile	Median	Mean	Third quartile	Maximum
A	1992	1996	2002	2001	2006	2009
B	1991	1992	1995	1998	2007	2009
C	1991	1999	2004	2003	2008	2010
D	1991	1999	2003	2003	2007	2010
E	1992	1998	2002	2002	2006	2010
F	1991	1997	2001	2000	2003	2009
G	1992	1996	2001	2000	2003	2006
H	1991	1995	1999	1999	2003	2006

Table A.6 *Summary of the data used for model validation per zone over time: the Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).*

	Sampling year					
Zone	**Minimum**	**First quartile**	**Median**	**Mean**	**Third quartile**	**Maximum**
A	1992	1996	2002	2001	2006	2009
B	1991	1994	1996	1999	2007	2009
C	1991	2000	2004	2003	2008	2010
D	1991	2000	2004	2003	2008	2010
E	1992	2001	2004	2003	2007	2010
F	1991	1996	2002	2000	2004	2009
G	1992	1997	2001	2000	2004	2006
H	1991	1997	2002	2000	2003	2006

Table A.7 *Spearman correlation analysis between the PCB time trends found based on model 3 and the PCB emission estimated by Breivik et al. (2007). The Atlantic zone (A), the Flemish banks (B), the coastal zone (C), and the Scheldt zone (D), the mouth of the Scheldt (E) and three trajectories from Vlissingen to Hansweert (F), from Hansweert to the Dutch-Belgian border (G) and from the border to Antwerp (H).Significance codes: 0.01 '*'.*

	CB28	**CB31**	**CB52**	**CB101**	**CB118**	**CB138**	**CB105**	**CB153**	**CB156**	**CB180**
A	0.91*	0.91*	0.91*	0.91*	0.91*	0.91*	0.91*	0.91*	0.91*	0.91*
B	0.74*	0.74*	0.74*	0.74*	0.74*	0.74*	0.74*	0.74*	0.74*	0.74*
C	0.89*	0.89*	0.89*	0.89*	0.89*	0.89*	0.89*	0.89*	0.89*	0.89*
D	0.86*	0.87*	0.87*	0.87*	0.86*	0.86*	0.86*	0.86*	0.86*	0.86*
E	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.34
F	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
G	-0.26	-0.25	-0.25	-0.25	-0.25	-0.26	-0.25	-0.25	-0.25	-0.25
H	0.51	0.51	0.51	0.52	0.51	0.51	0.51	0.51	0.51	0.51

B

Supportive information for chapter 3

Figures

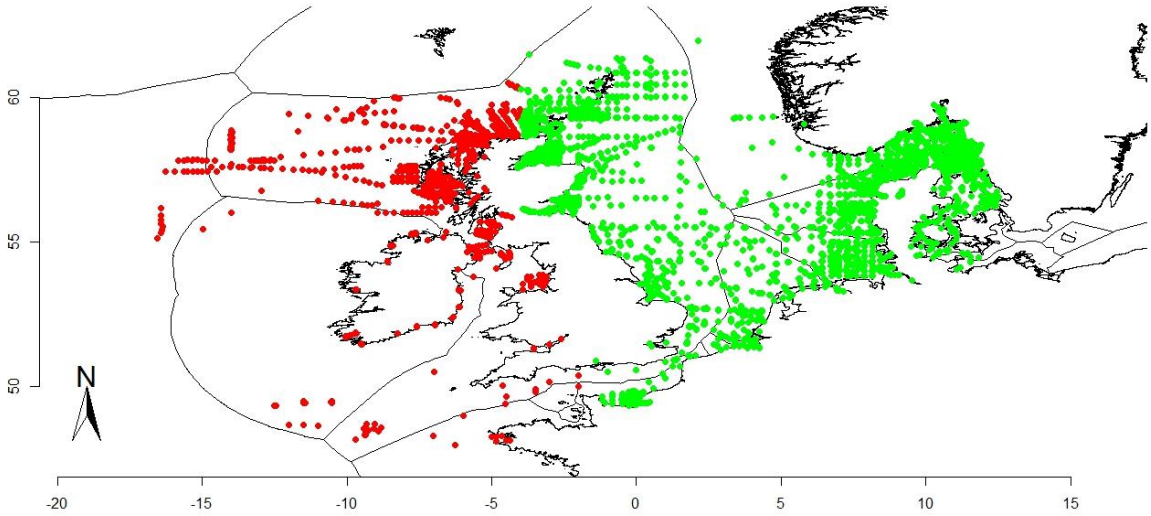


Figure B.1 *Spatial distribution of the chlorophyll a data in the greater North Sea (green dots) and the Celtic Sea (including west of Scotland; red dots). Information about the data selection is listed in Table B.1.*

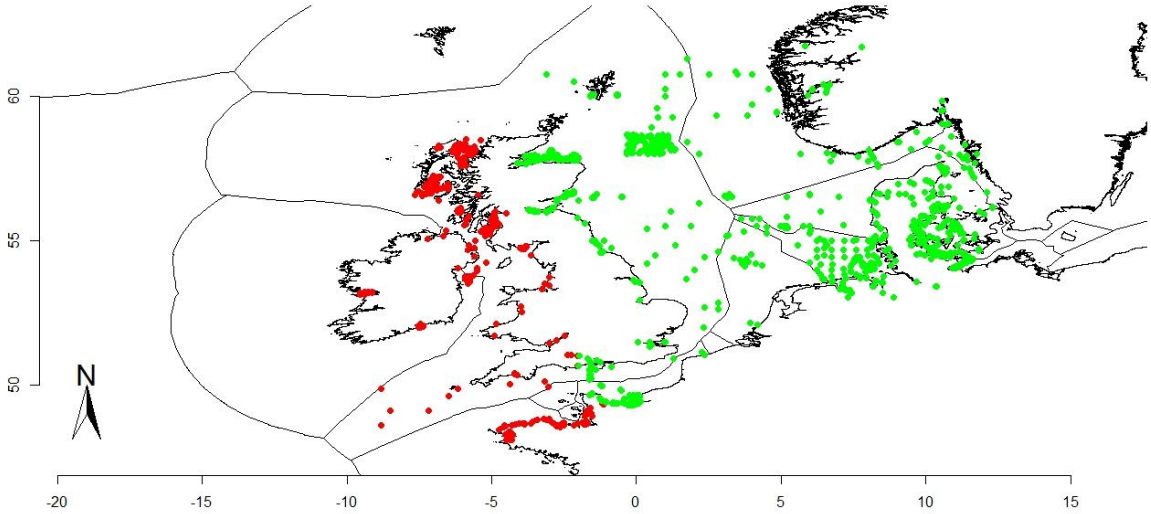


Figure B.2 *Spatial distribution of the sediment organic carbon data in the greater North Sea (green dots) and the Celtic Sea (including west of Scotland; red dots)**.** Information about the data selection is listed in Table B.3.*

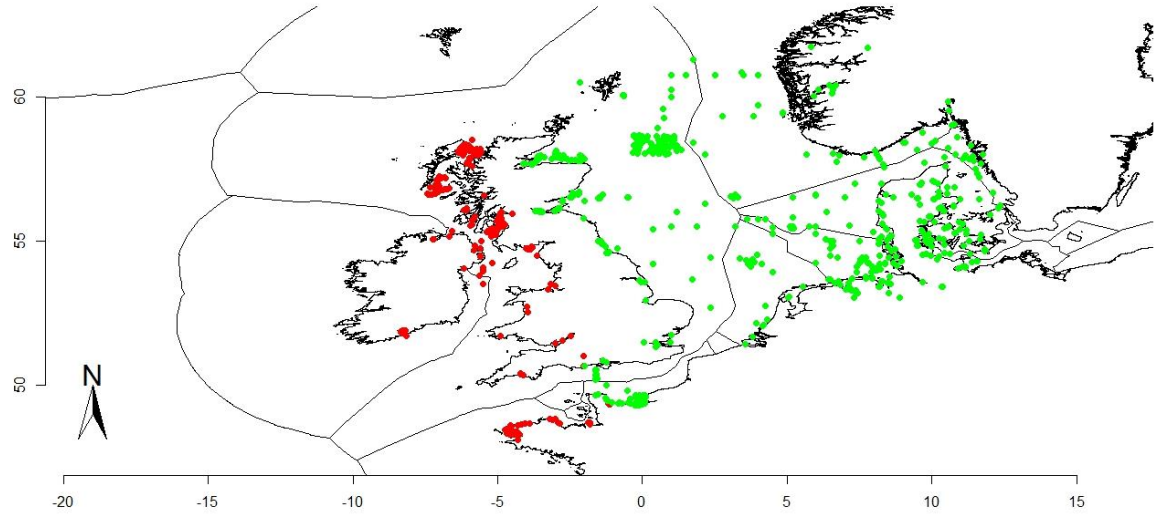


Figure B.3 *Spatial distribution of the PCB sediment data in the greater North Sea (green dots) and the Celtic Sea (including west of Scotland; red dots).* ***Information about the data selection is listed in Table B.2.***

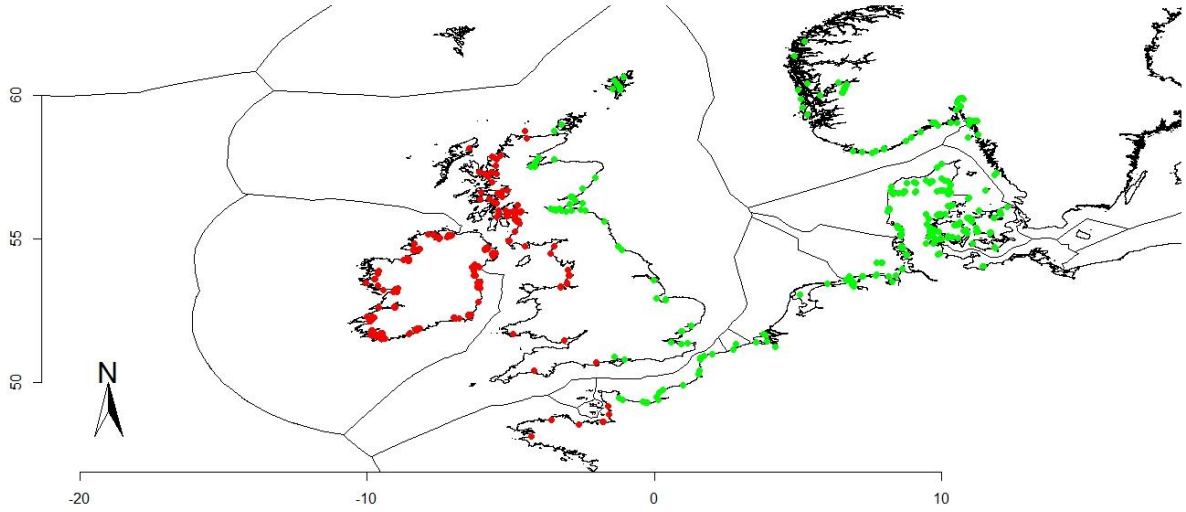


Figure B.4 *Spatial distribution of the PCB mussel data in the greater North Sea (green dots) and the Celtic Sea (including west of Scotland; red dots).* ***Information about the data selection is listed in Table B.2.***

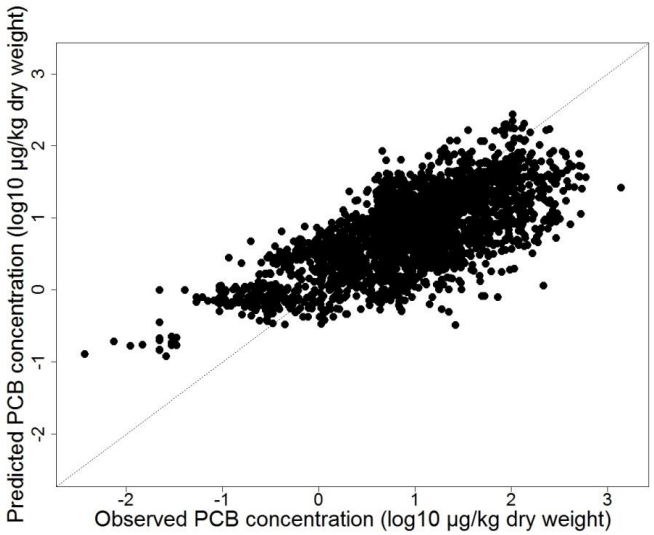


Figure B.5 *Application of the spatiotemporal model (model configuration 4) for the PCB concentrations in mussels on the training data (n = 2744). The estimated PCB concentrations in mussels are compared with the observed concentrations. The dotted line represents the first bisector and the correlation coefficient between predicted and observed values is 0.70 ($p < 2.0 \times 10^{-16}$). The adjusted r-squared values of the spatiotemporal PCB trends obtained (0.52 and 0.49 in sediment and in mussels, respectively; Table 1) indicate a good model fit, but residual diagnostics show that low PCB concentrations were predicted too high.*

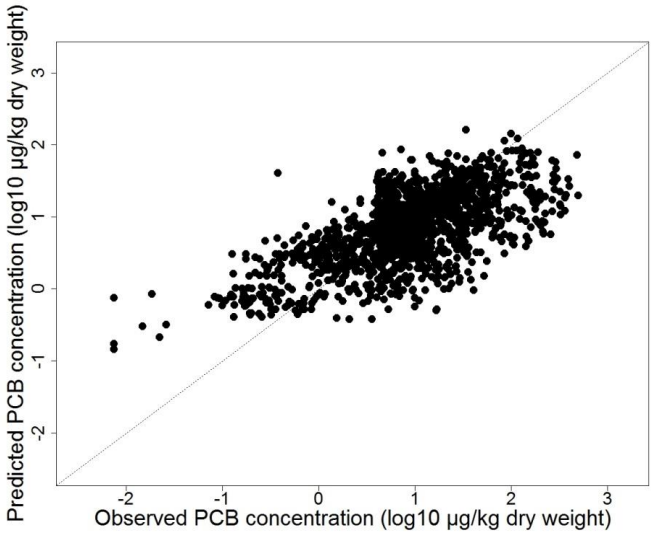


Figure B.6 *Application of the spatiotemporal model (model configuration 4) for the PCB concentrations in mussels on the validation data (n = 1372). The estimated PCB concentrations in mussels are compared with the observed concentrations. The dotted line represents the first bisector and the correlation coefficient between predicted and observed values is 0.66 ($p < 2.0 \times 10^{-16}$). The adjusted r-squared values of the spatiotemporal PCB trends obtained (0.52 and 0.49 in sediment and in mussels, respectively; Table 1) indicate a good model fit, but residual diagnostics show that low PCB concentrations were predicted too high.*

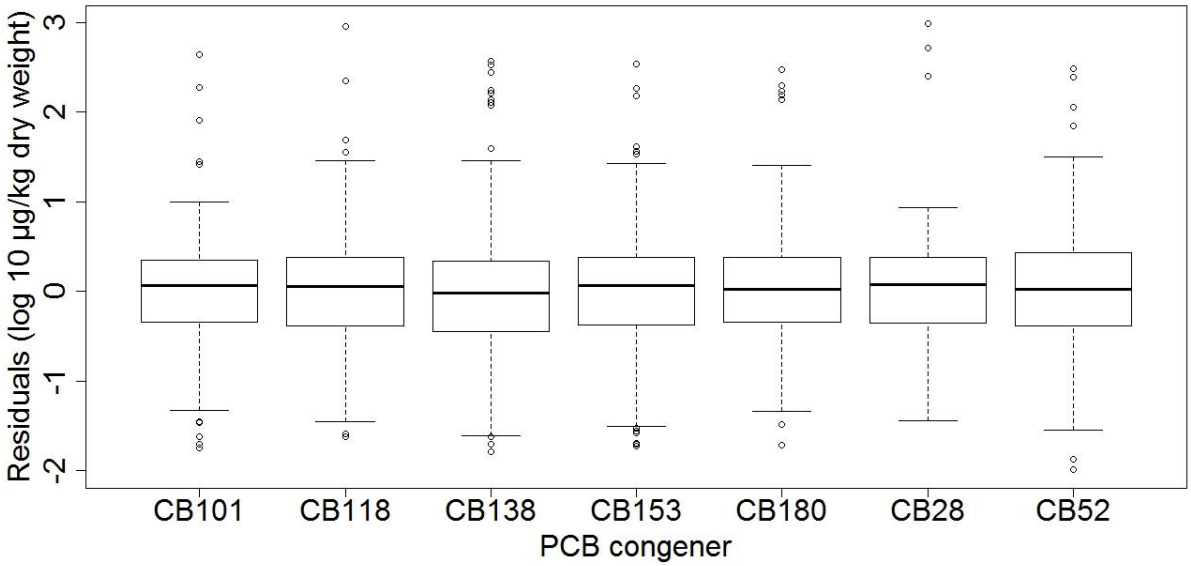


Figure B.7 *Residuals of the spatiotemporal trend model for PCB concentrations in sediment plotted against the seven congeners that were used in the present paper (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) by means of box-and-whisker plots.*

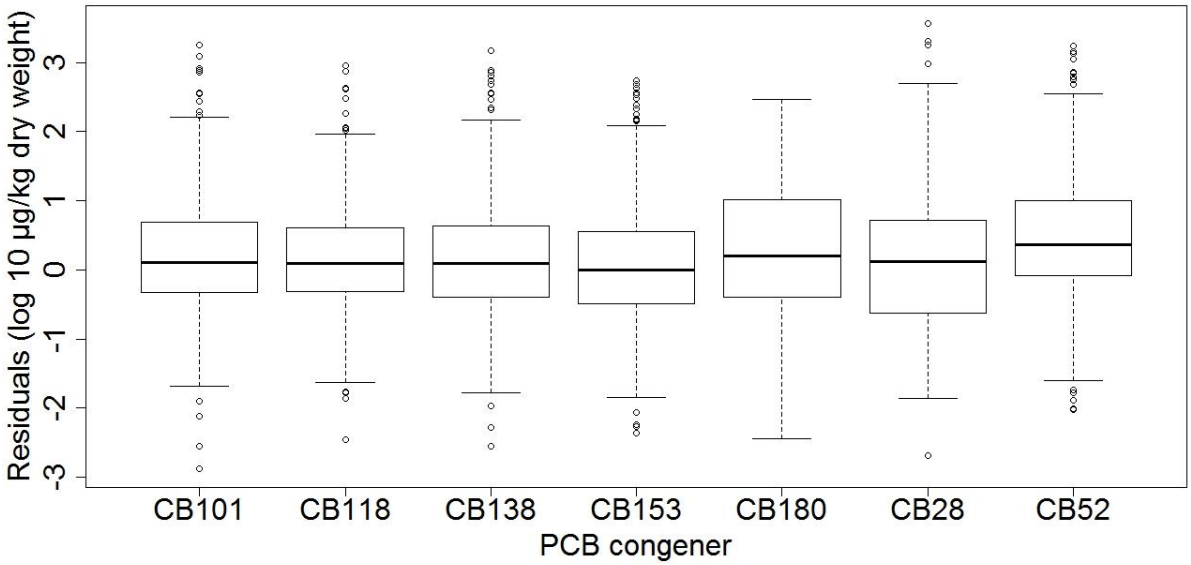


Figure B.8 *Residuals of the spatiotemporal trend model for the PCB concentrations in mussels plotted against the seven congeners that were used in the present paper (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) by means of box-and-whisker plots.*

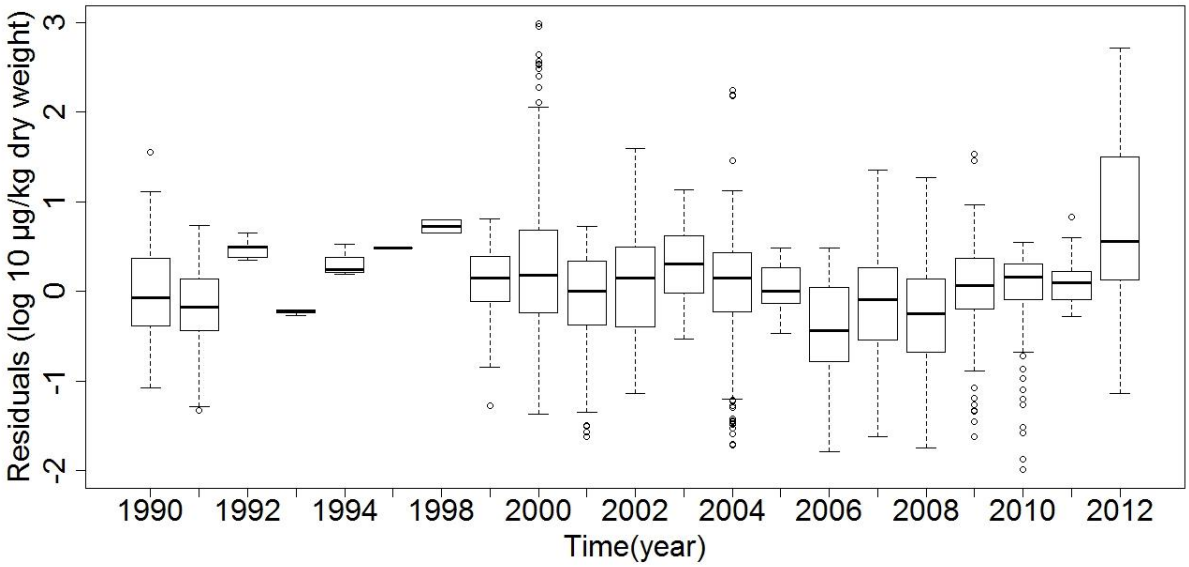


Figure B.9 *Residuals of the spatiotemporal trend model for the PCB concentrations in sediment plotted against the sampling years by means of box-and-whisker plots.*

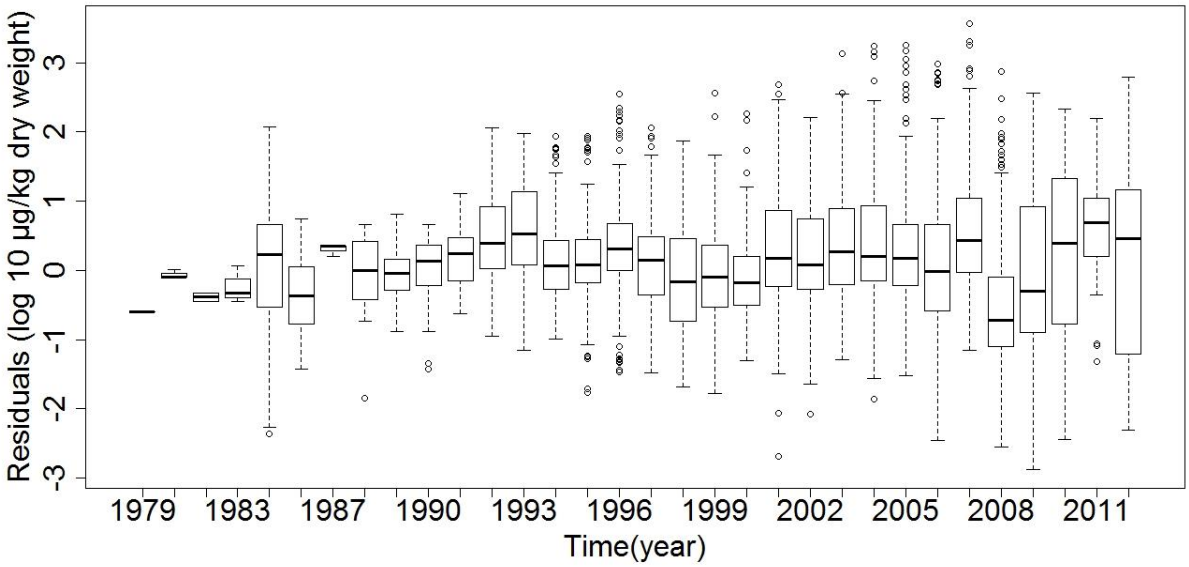


Figure B.10 *Residuals of the spatiotemporal trend model for the PCB concentrations in mussels plotted against the sampling years by means of box-and-whisker plots.*

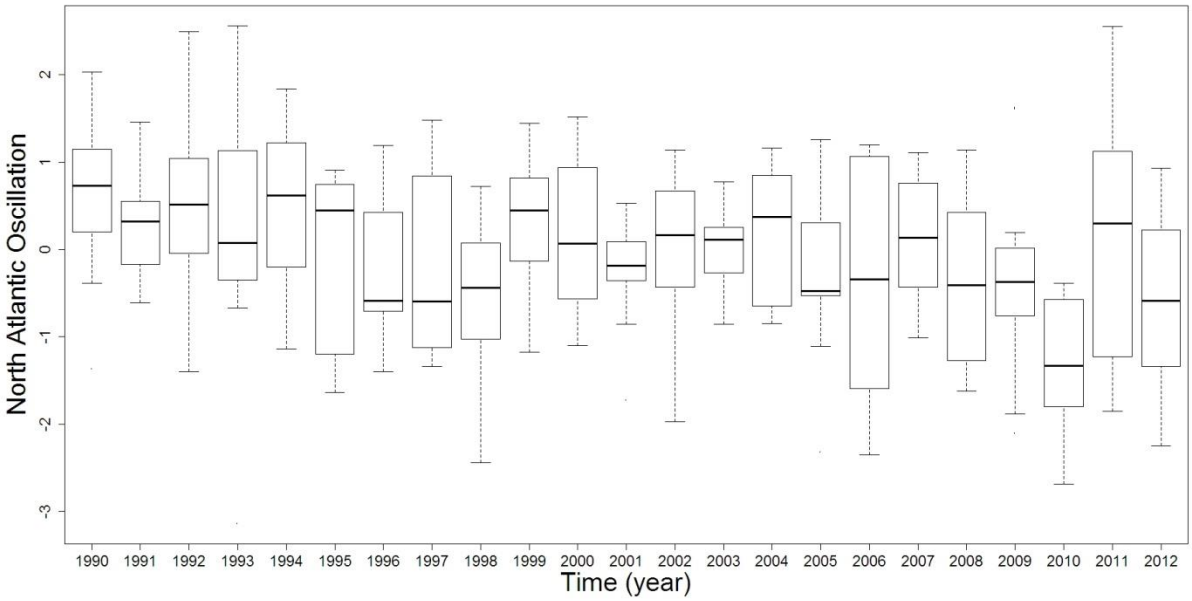


Figure B.11 *Yearly averages of the North Atlantic Oscillation between 1990 and 2012 plotted by means of box-and-whisker plots.*

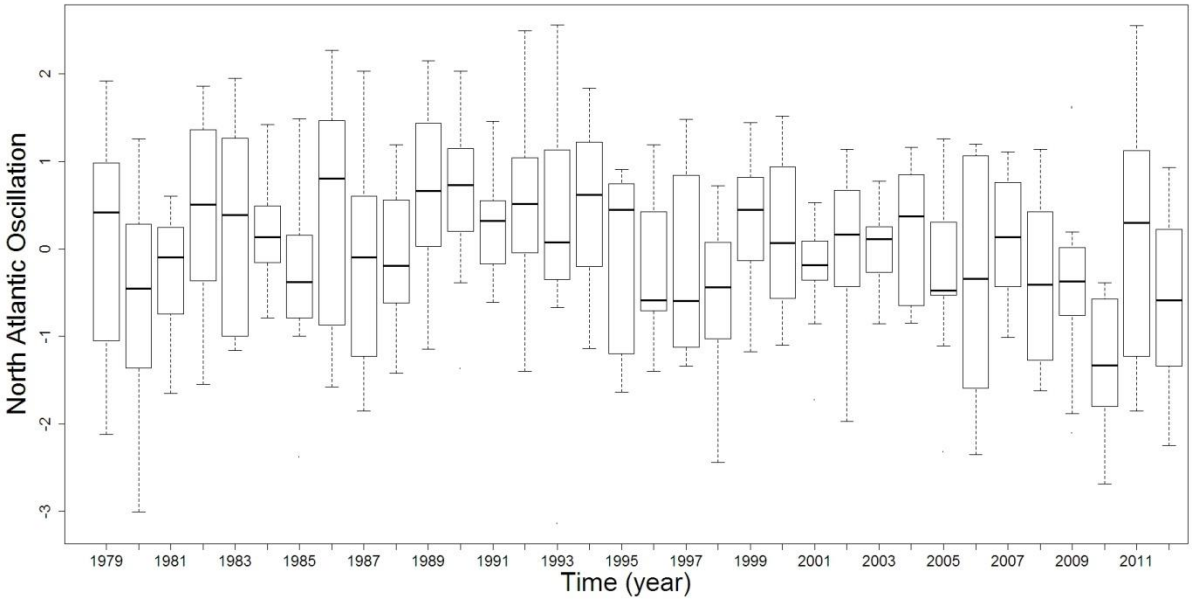


Figure B.12 *Yearly averages of the North Atlantic Oscillation between 1979 and 2012 plotted by means of box-and-whisker plots.*

Tables

Table B.1 *Selection criteria for CHFLa concentrations. Search in ICES database, accessed on 3rd June 2014. The search resulted in 703 data (of which 440 in the North Sea and 263 in the Celtic Sea) collected between 1975 and 2012.*

In ICES	**Amount of data**
Marine data	
Oceanographic data	281,537,169
Per parameter	
CHFLa	342,672
Quality control	
Avoid detection limits	339,571
Region	
Greater North Sea	
Celtic Sea including west of Scotland	198,383
Depth	
<3m	
or pressure	
< 1.29 bar	46,005
Averaging	
For each unique combination of zone, year and month	703

Table B.2 *Selection criteria for PCB concentrations in sediment and mussels. Search in ICES database, accessed on 3rd June 2014. The search resulted in 4,116 data of PCB concentrations in mussels and 2,185 data of PCB concentrations in sediment collected between 1979 and 2012.*

In ICES	**Amount of data**
Marine data	
Contaminants and biological effects	11,549,169
Per parameter	
7PCBs (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180)	99,805
Further refining	
Quality control	
Avoid detection limits	72,272
Region	
Greater North Sea	
Celtic Sea including west of Scotland	56,498
Select matrix	
< 2000µm fraction	13,045 (sediment data)
Whole soft body	7,936 (mussel data)
Depth	
<0.02m	3,080 (sediment data)
	7,936 (mussel data)
Averaging	
For each unique combination of zone, year, month and distance to the coastline	4,116 (mussel data) 2,185 (sediment data)

Table B.3 *Selection criteria for OC sediment concentrations. Search in ICES database, accessed on 3rd June 2014. The search resulted in 224 data (of which 146 in the North Sea and 78 in the Celtic Sea) collected between 1986 and 2012.*

In ICES	**Amount of data**
Marine data	
Contaminants and biological effects	11,549,169
Per parameter	
OC	27,065
Quality control	
Avoid detection limits	26,795
Region	
Greater North Sea	
Celtic Sea including west of Scotland	11,457
Depth	
<0.02m	6,816
Matrix	
Sediment on dry weight basis	3,599
Conversion to g/100g dry weight	3,599
Averaging	
For each unique combination of zone, year and month	224

Table B.4 *Selection criteria for CB28 aqueous concentrations. Search in ICES database, accessed on 1st October 2014. The search resulted in 151 data (of which 150 in the North sea and 1 in the Celtic Sea) collected between 2005 and 2011.*

In ICES	**Amount of data**
Marine data	
Contaminants and biological effects	11,549,169
Per parameter	
CB28	42,377
Per matrix	
Sea water sample-before filtration	455
Further refining	
Region	
Greater North Sea	
Celtic Sea including west of Scotland	402
Quality control	
Avoid detection limits	153
Depth	
5.5m	151

C

Supportive information for chapter 5

Figures

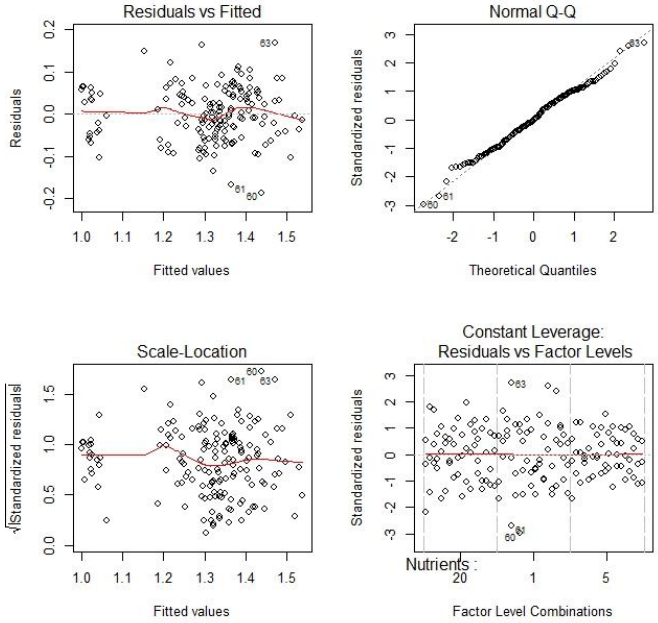


Figure C.1 *Residual diagnostics for model configuration 1. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality, and Panel D shows the standardised residuals versus leverage and the Cook statistic is superimposed as contour plots.*

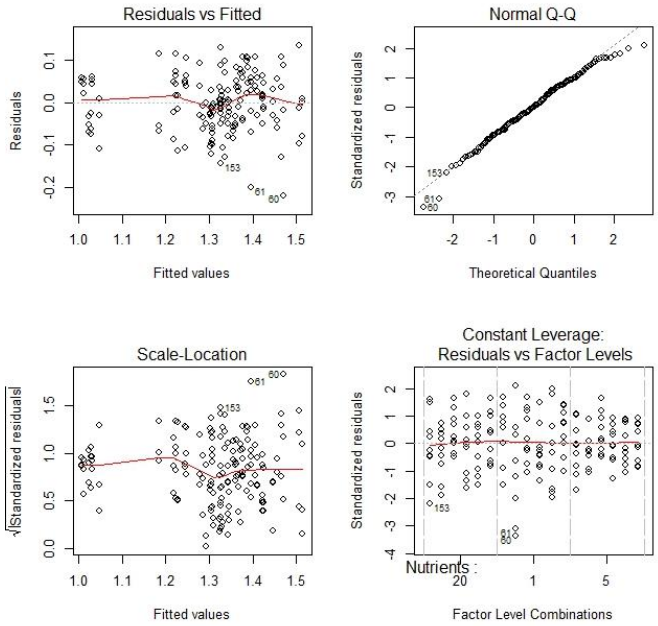


Figure C.2 *Residual diagnostics for model configuration 2. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality, and Panel D shows the standardised residuals versus leverage and the Cook statistic is superimposed as contour plots.*

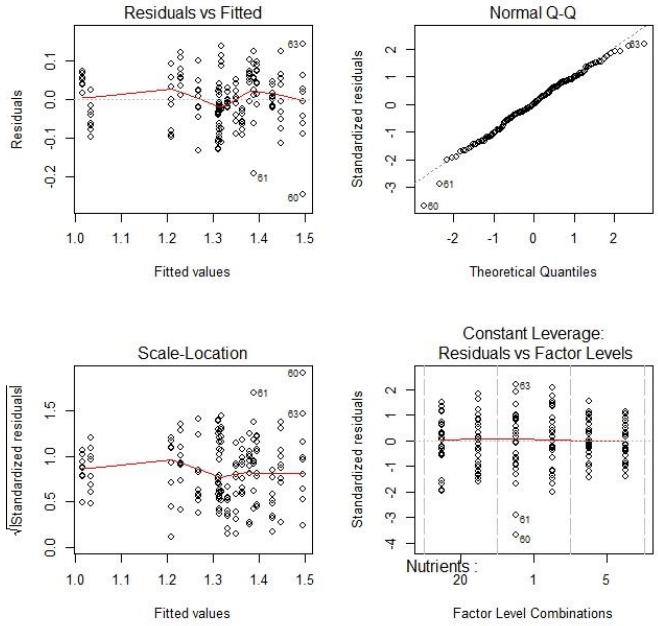


Figure C.3 *Residual diagnostics for model configuration 3. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality, and Panel D shows the standardised residuals versus leverage and the Cook statistic is superimposed as contour plots.*

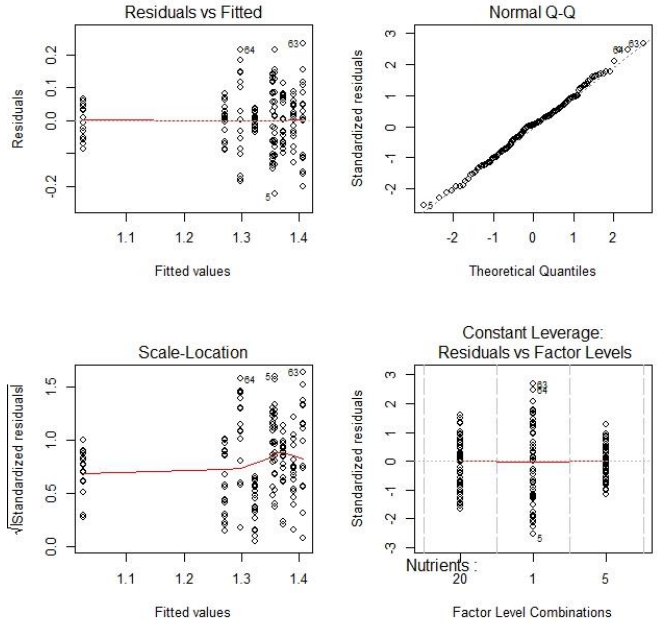


Figure C.4 *Residual diagnostics for model configuration 4. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality, and Panel D shows the standardised residuals versus leverage and the Cook statistic is superimposed as contour plots.*

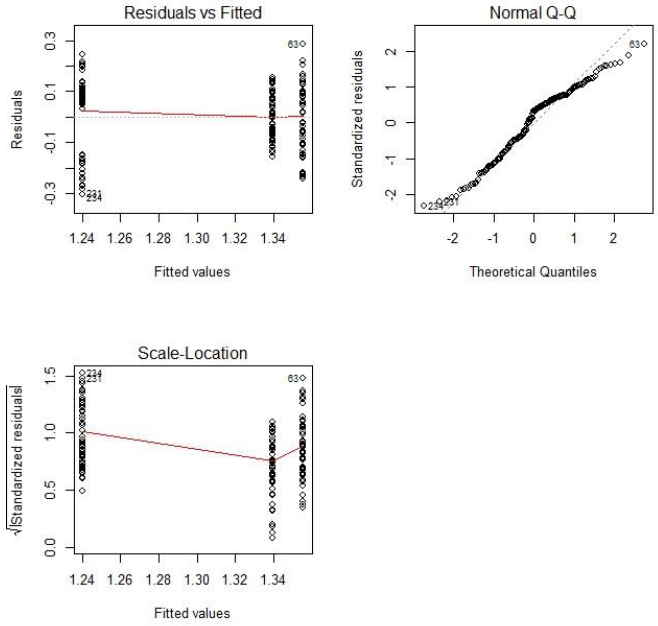


Figure C.5 *Residual diagnostics for model configuration 5. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality. The standardised residuals versus leverage and the Cook statistic could not be plotted.*

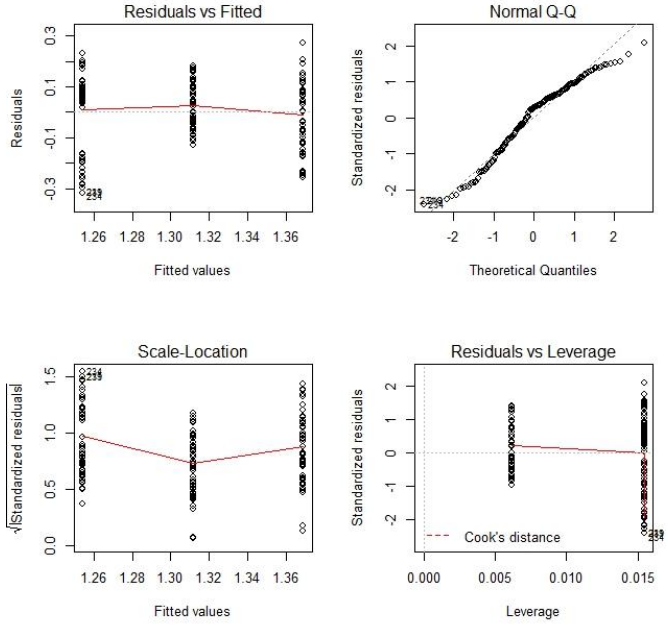


Figure C.6 *Residual diagnostics for model configuration 6. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality, and Panel D shows the standardised residuals versus leverage and the Cook statistic is superimposed as contour plots.*

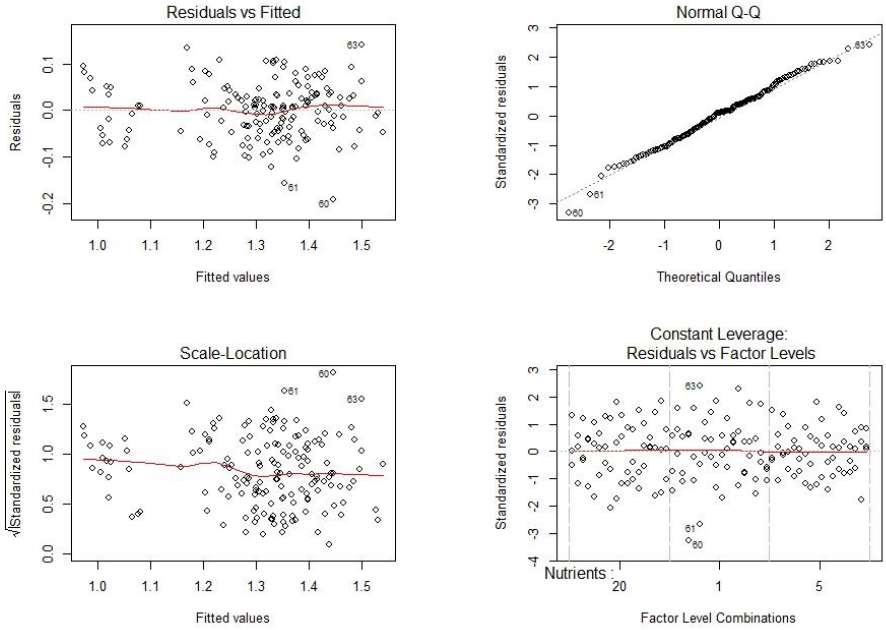


Figure C.7 *Residual diagnostics for model configuration 7. Model validation graphs obtained by applying a linear regression model on the experimental data obtained in the algal growth inhibition test. Panel A and C show residuals versus fitted values. Panel B is a QQ-plot for normality, and Panel D shows the standardised residuals versus leverage and the Cook statistic is superimposed as contour plots.*

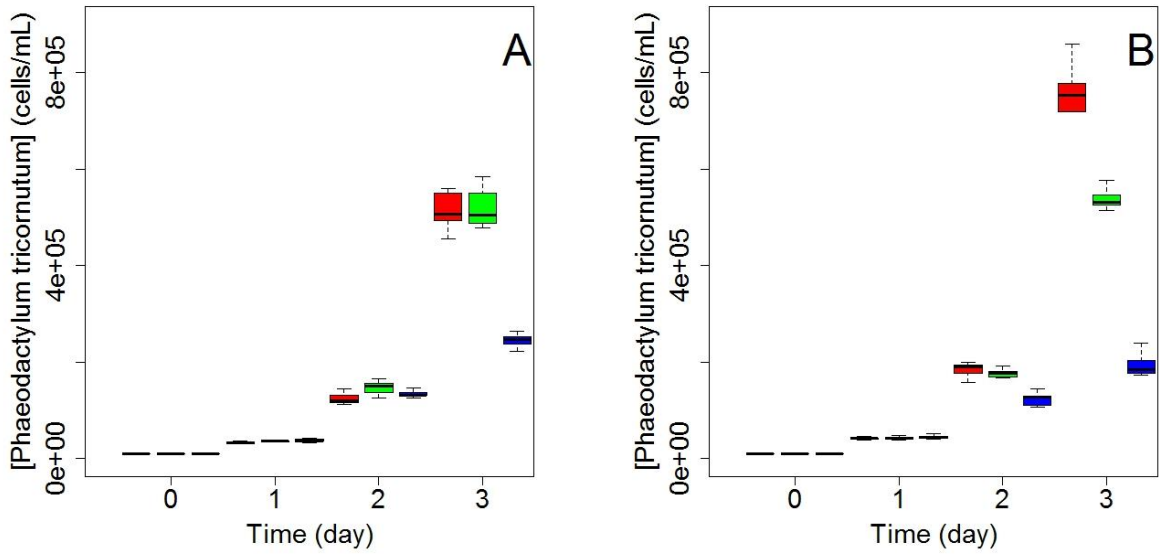


Figure C.8 *Two-panel boxplot illustrating the evolution of Phaeodactylum tricornutum concentrations (cells.mL^{-1}) in the 72h algal growth inhibition test for two incubation temperatures: 16°C (A) and 23°C (B). For each incubation temperature, three different nutrient regimes are plotted: 14 µmol P.L^{-1} & 588 µmol N.L^{-1} (red), 2.8 µmol P.L^{-1} & 120 µmol N.L^{-1} (green) and 0.7 µmol P.L^{-1} & 30 µmol N.L^{-1}(blue). Each single boxplot covers three chemical exposure and three light intensities.*

Tables

Table C.1 *Oceanographic characteristics at the sampling location along the Belgian coast just outside the harbour of Zeebrugge (MOW1). Measurements represent the conditions at the moment that the PDMS sheets were collected on March 27th, 2014.*

	Latitude	**Longitude**	**Incubation temperature**	**pH**	**Turbidity**	**Salinity**
MOW1	51° N 21.644’	3° E 6.992’	7.98°C	8.40	24.5 FTU	30.47 PSU

Table C.2 *The pH of the test medium measured at the start and end of the test period.*

	Blank control	**Growth control**	**Chemical exposure**
Start of experiment	7.92	7.92	7.92
End of experiment	7.90 ± 0.03	7.91 ± 0.04	7.91 ± 0.03

Table C.3 *Detailed overview of the hierarchical backward elimination model selection method. Seven different model configurations were tested and for each configuration we reported the Akaike information criterion (AIC), the adjusted correlation coefficient (R^2) and the variability explained by the covariates that were included (Var. explained). For each model configuration, we used the F-statistic and its associated p-value to asses which covariates contributed to the model at a 5% level of significance. Covariates considered were: the moment of sampling (DAY), the nutrient regime (NUT), the incubation temperature (TEM), the chemical exposure (ORC) and the light intensity (LIG). If appropriate quadratic effects (^{2}) and interaction terms (:) were included.*

	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**	**Model 7**
Main effects							
DAY	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
DAY²	< 0.001	< 0.001	< 0.001	0.006	0.060		< 0.001
NUT	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
TEM	< 0.001	< 0.001	< 0.001				< 0.001
LIG	0.020	0.011					0.007
ORC	0. 126						0. 126
Interaction effects							
DAY:NUT	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
DAY²:NUT	0.380	0.390	0.390	0.580			0.390
DAY:TEM	< 0.001	< 0.001	< 0.001				< 0.001
DAY²:TEM	0.450	0.460	0.460				0.460
DAY:LIG	0.420	0.421					0.398
DAY²:LIG	0.910	0.913					0.905
DAY:ORC	0.340						
DAY²:ORC	0.780						
ORC:NUT							0.25
ORC:TEM							0.08
ORC:LIG							0.04
Model fit							
Var. explained	88.1	87.0	85.2	66.6	12.8	10.7	90.8
AIC	-387.2	-392.4	-392.3	-309.9	-192.8	-191.2	-397.0

Table C.4 *Freely dissolved concentrations of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) measured at sampling location MOW1 along the Belgian coast. We report the concentrations of seven individual PCB congeners (ICES-PCBs) as well as their total (∑) concentrations. For the PAHs focus was on 16 EPA PAHs except for naphthalene. Detection limit for PCBs and PAHs was 1.0 ng.L^{-1} and 2.0 ng.L^{-1}, respectively. Concentrations found were compared to the environmental quality standards as reported in the Marine strategy Framework Directive. Measured concentrations as well as the environmental quality standards are reported in ng.L^{-1}. Organic chemicals below the detection limit were indicated as not detected (ND). In addition, substance-specific PCB and PAH concentrations (in ng.L^{-1}) observed close to the harbour of Zeebrugge sampled in 2010 and reported by Monteyne et al. (2013) are listed.*

	Chemical exposure	**Observations by Monteyne et al. (2013) in 2010**	**Environmental quality standard**
PCBs			
PCB 28	1.5 ± 0.5	0.09	-
PCB 52	2.0 ± 0.3	0.23	-
PCB 101	2.0 ± 0.1	0.21	-
PCB 118	ND	0.09	-
PCB 138	1.3 ± 0.6	0.14	-
PCB 153	ND	0.17	-
PCB 180	ND	0.03	-
$\sum PCB_7$	6.8 ± 1.5	0.96	2
PAHs			
Acenaphthylene	ND	1.00	4000
Acenaphthene	6.8 ± 0.2	7.90	60
Fluorene	2.4 ± 0.1	2.50	2000
Phenanthrene	24.9 ± 8.5	3.10	100
Anthracene	ND	0.47	100
Fluoranthene	5.5 ± 1.1	5.20	100
Pyrene	ND	3.50	40
Benzo(a)anthracene	ND	0.21	300
Chrysene	ND	0.37	1000
Benzo(b)fluoranthene	ND	0.20	30
Benzo(k)fluoranthene	ND	0.07	30
Benzo(a)pyrene	ND	0.21	50
Indeno(1,2,3-c,d)pyreen	ND	0.04	2
Dibenzo(a,h)anthracene	ND	0.01	500
Benzo(g,h,i)perylene	ND	0.05	2
$\sum PAH_{15}$	39.7 ± 9.8	24.8	-

D

Supportive information for chapter 6

Figures

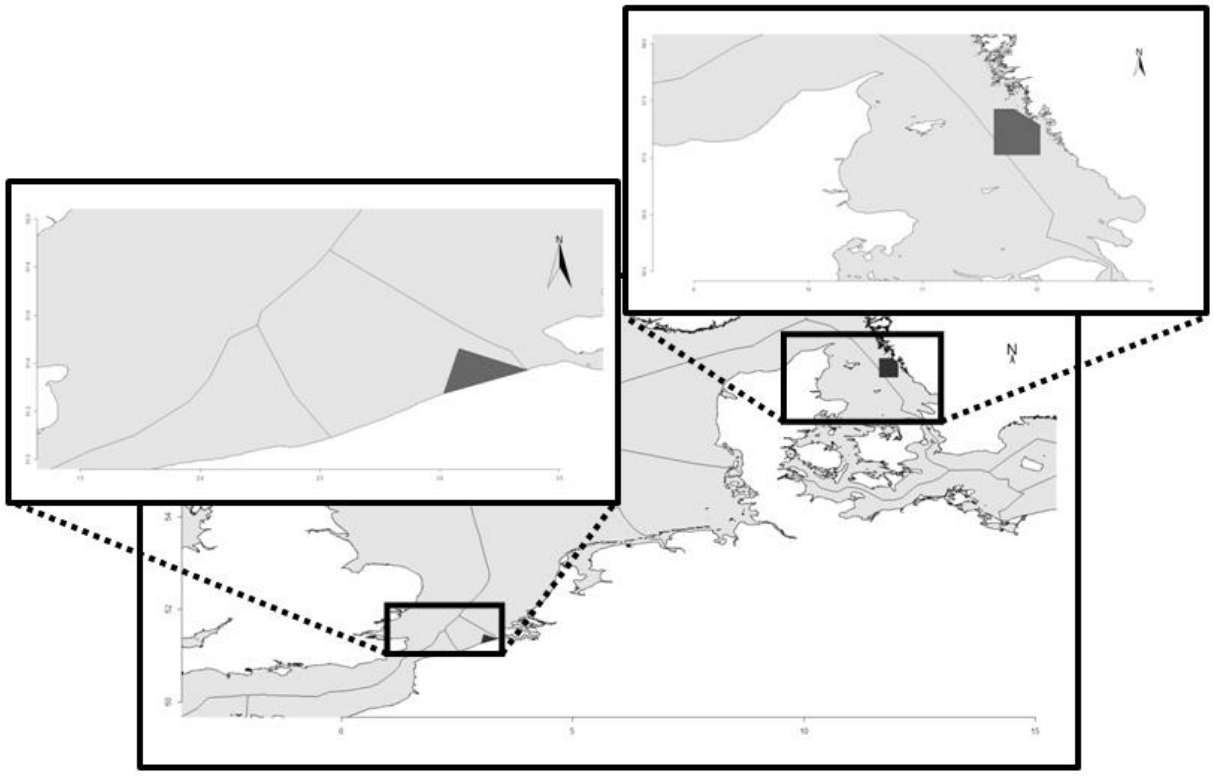


Figure D.1 *Two study regions selected in the Kattegat (strait between North Sea and Baltic Sea) and in the Belgian part of the North Sea. The study area in Belgian waters is 233 km² large, is delimited by 51.37 °N, 3.37 °E; 51.27 °N, 3.02 °E; 51.46 °N, 3.08 °E and is located in the proximity of Belgian sampling locations "140", "700","780","710_a". The study area in Kattegat is located at the Swedish west coast, has an area of 1144 km², is delimited by 57.02 °N, 11.63 °E; 57.42 °N, 11.63 °E; 57.42 °N, 12.03 °E; 57.03 °N, 12.03°E and is in the proximity of Swedish sampling location "Fladen".*

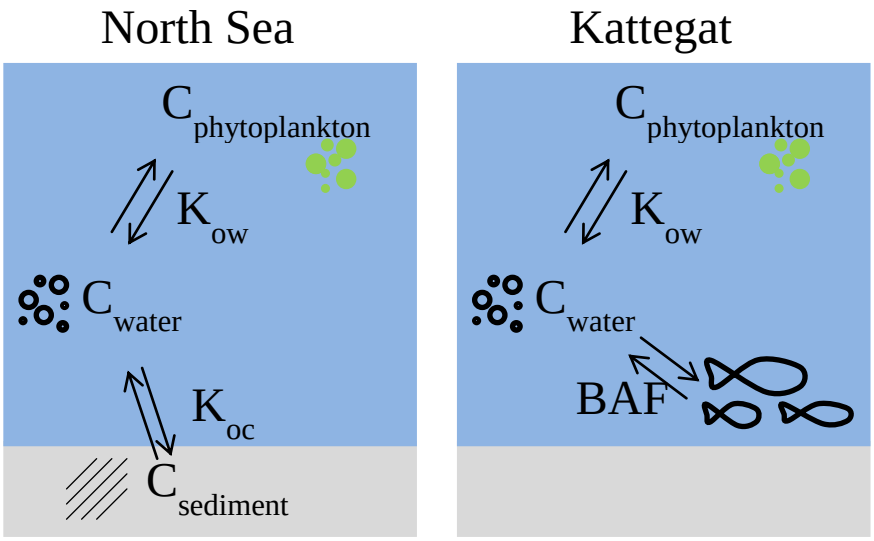


Figure D.2 *Conversion between the different environmental compartments. The organic carbon – water partition coefficient (K_{oc}), the octanol-water partition coefficient (K_{ow}) and bioaccumulation factor (BAF) were used to convert the sediment concentrations ($C_{sediment}$) and biotic concentrations to internal phytoplankton lipid weight concentrations ($C_{phytoplankton}$).*

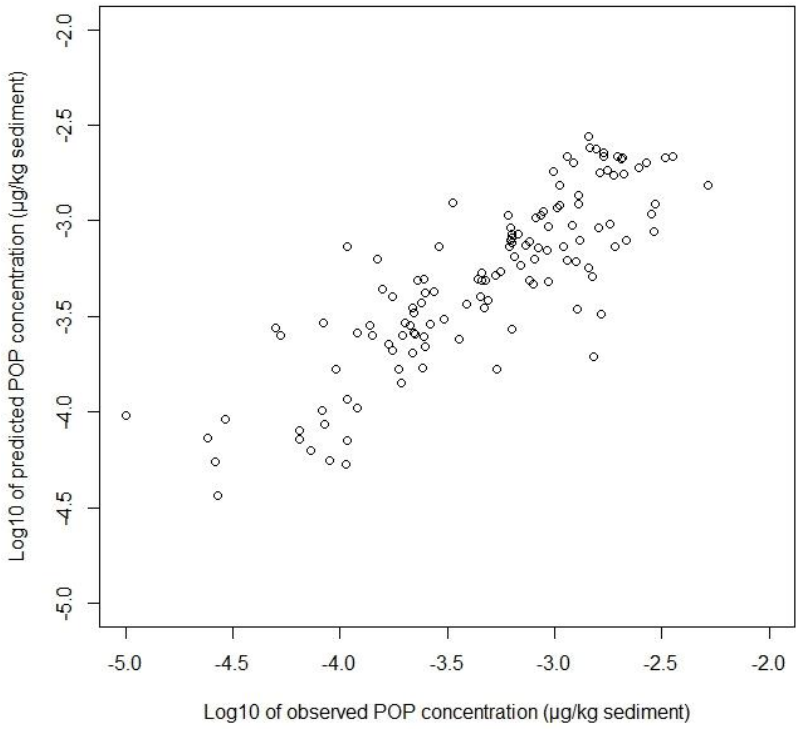


Figure D.3 *Implementation of the additive model for the PCB and pesticide concentration on independent data from the Belgian continental zone.*

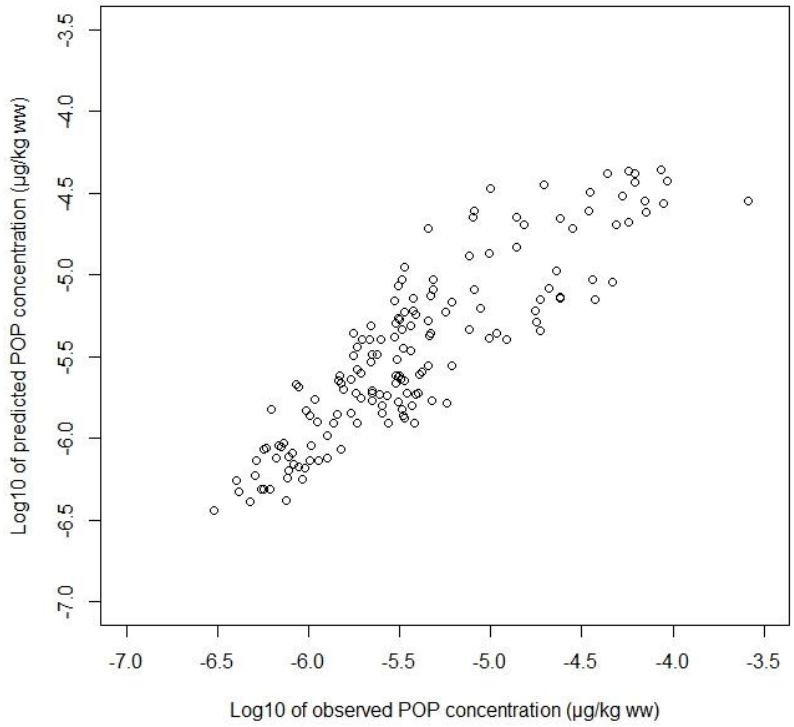


Figure D.4 *Implementation of the additive model for the PCB and pesticide concentration on independent data from the Kattegat region.*

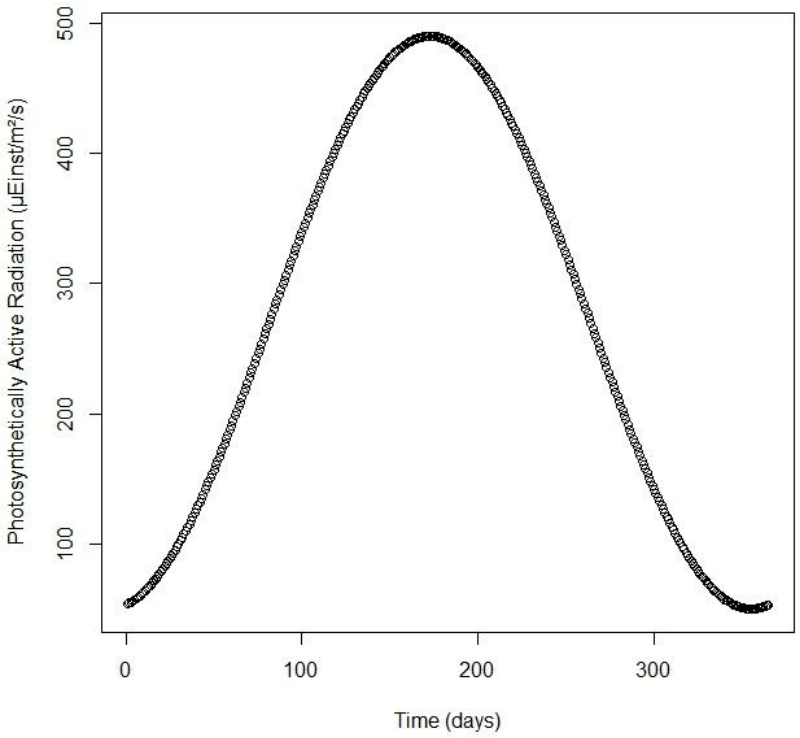


Figure D.5 *The amount of photosynthetically active radiation was calculated based on an empirical function*

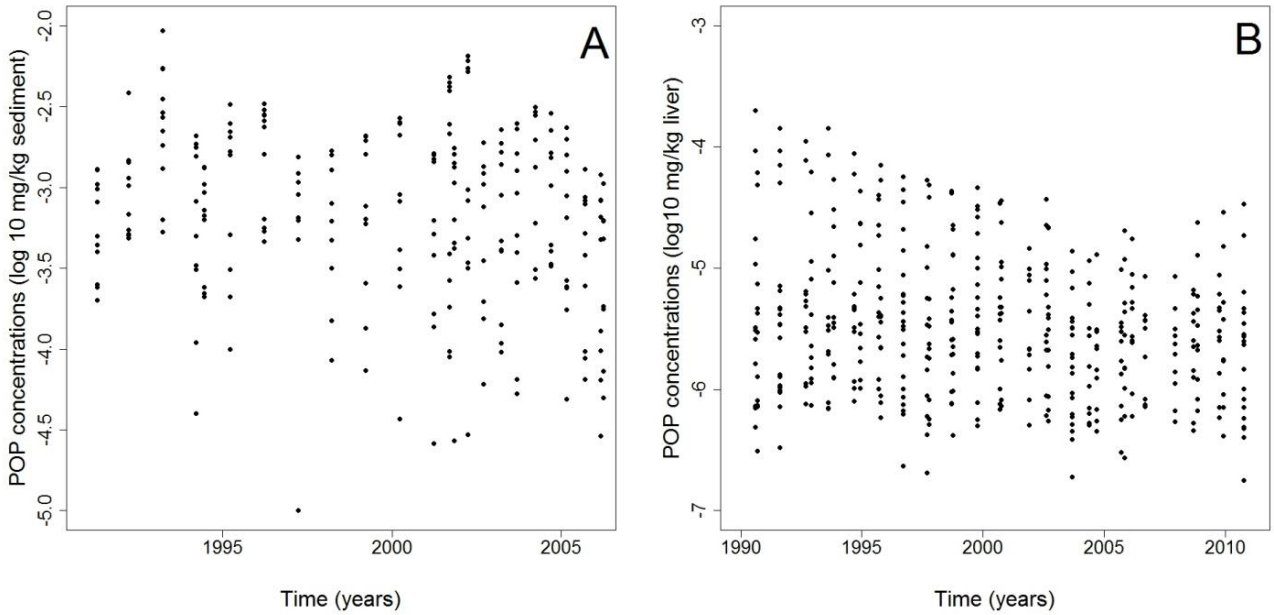


Figure D.6 *POP concentrations in the sediment in the Belgian continental zone (A) and in mussel liver in the Kattegat (B).*

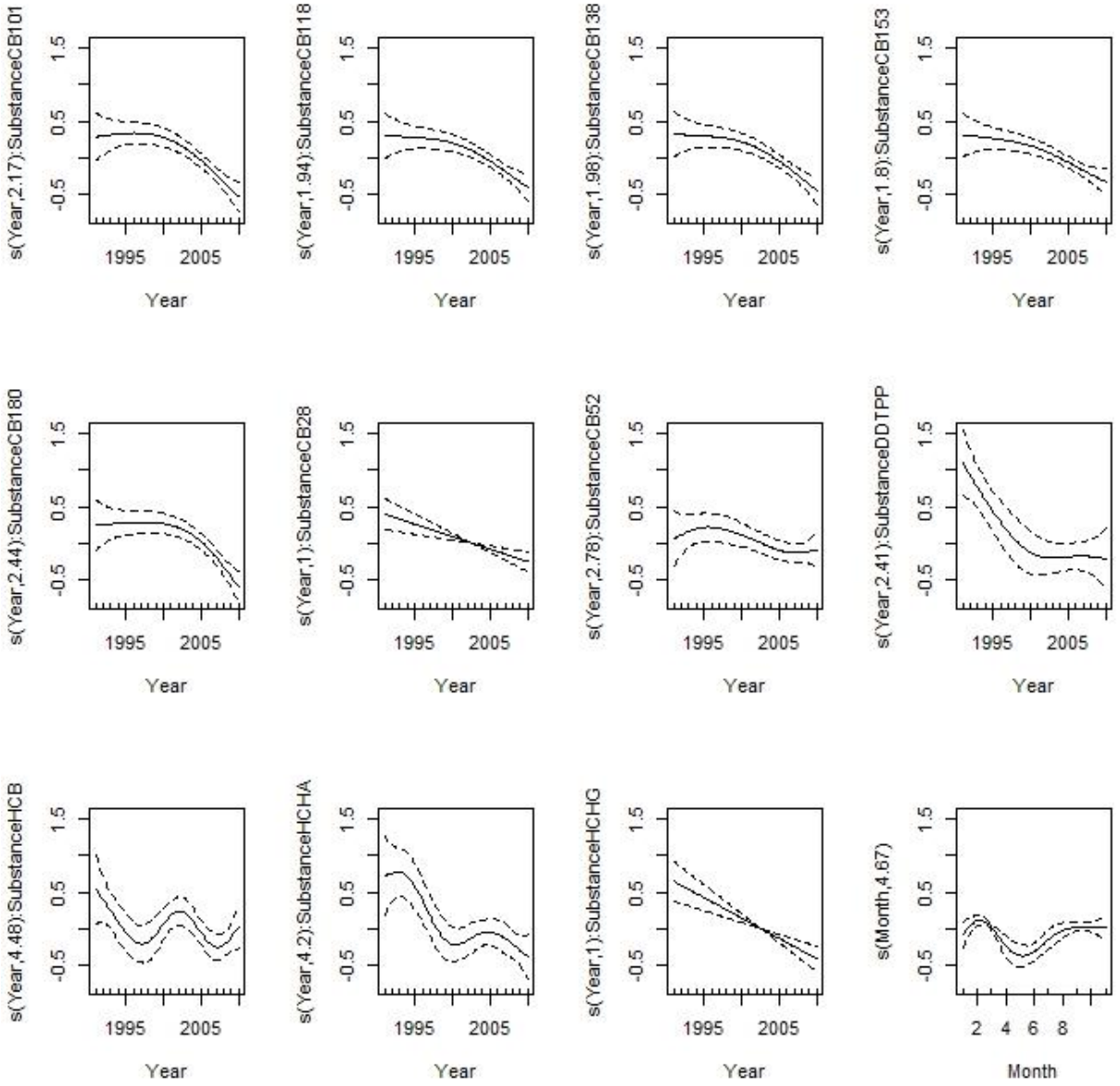


Figure D.7 *Smoothers of additive model for the PCB and pesticide concentration in the Belgian continental zone*

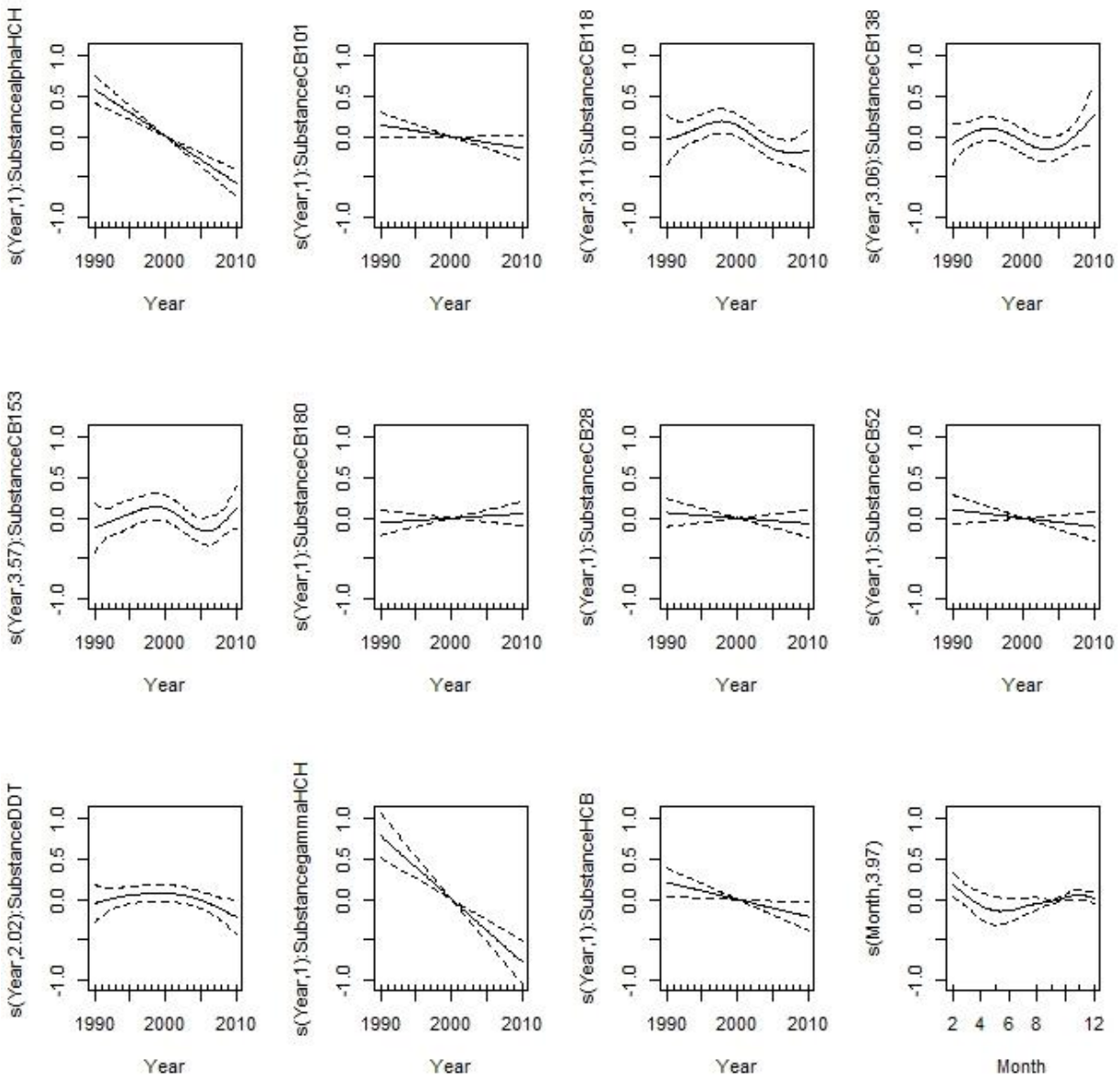


Figure D.8 *Smoothers of additive model for the PCB and pesticide concentration in the Kattegat region*

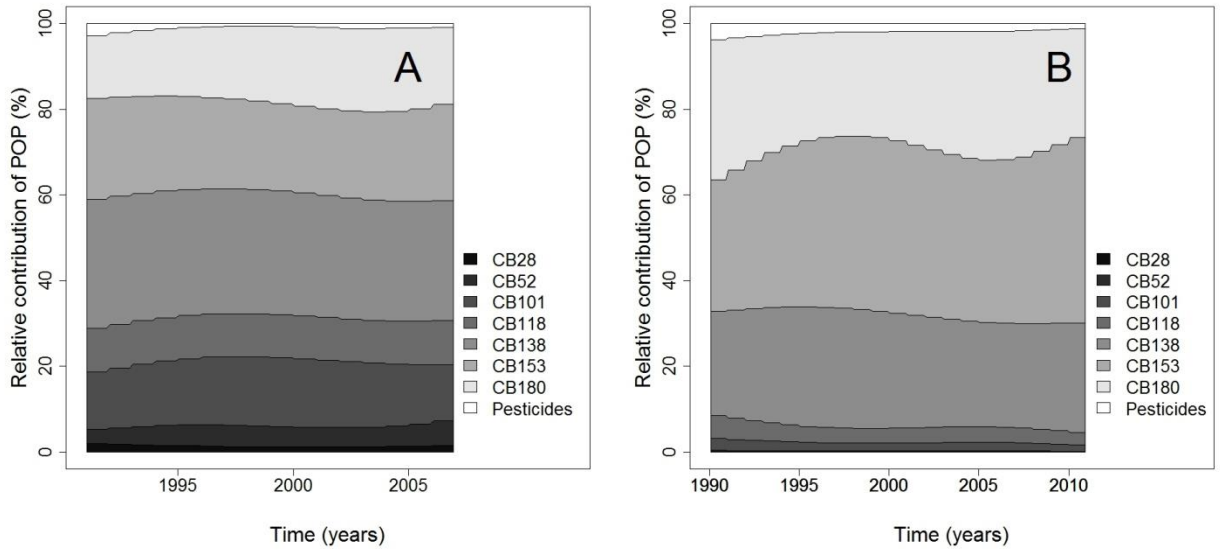


Figure D.9 *Evolution of POP mixture in the Belgian continental zone (A) and in the Kattegat (B).*

Tables

Table D.1 *Selection criteria for PCBs and pesticides in the Belgian coastal zone. Search in BMDC database, accessed on 1st October 2013.*

In BMDC	**Amount of data**
Parameters measured in sediment	
CB28	425
CB52	423
CB101	425
CB118	425
CB138	424
CB153	425
CB180	425
DDTPP	385
HCHA	389
HCHG	402
HCB	401
	Total: 4,549
Timing between 1991 and 2006 Measured in the sediment < 63µm Consider detection limits Region (233 km²) between 51.37 °N, 3.37 °E 51.27 °N, 3.02 °E 51.46 °N, 3.08 °E	Total: 708
Averaging for each unique combination of substance, year and month	
CB28	39
CB52	34
CB101	40
CB118	40
CB138	40
CB153	43
CB180	39
DDTPP	12
HCHA	31
HCHG	25
HCB	26
	Total: 369

Table D.2 *Selection criteria for physical-chemical variables in the Belgian coastal zone. Search in BMDC database, accessed on 1st October 2013.*

In BMDC	**Amount of data**
Parameters measured in sediment	
Nitrate	2,205
Nitrite	2,204
Ammonia	4,125
Phosphate	3,298
Temperature	3,847
Chlorophyll a	3,450
	Total: 19,130
Timing between 1991 and 2006 Measured 3 m below water surface Consider detection limits Region (233 km²) between 51.37 °N, 3.37 °E 51.27 °N, 3.02 °E 51.46 °N, 3.08 °E	Total: 803
Averaging for each unique combination of substance, year and month	
Nitrate	79
Nitrite	81
Ammonium	81
Phosphate	83
Temperature	64
Chlorophyll a	83
	Total: 471

Table D.3 *Selection criteria for PCBs and pesticides in the Kattegat. Search in ICES database, accessed on 1st October 2013.*

In ICES	**Amount of data**
Parameters measured in sediment	
CB28	6,067
CB52	6,040
CB101	6,002
CB118	6,136
CB138	6,148
CB153	6,129
CB180	6,133
DDTPP	2,583
HCHA	6,136
HCHG	6,652
HCB	6,780
	Total: 64,806
Timing between 1990 and 2010 Measured in the liver of cod Consider detection limits Region (1144 km²) between 57.02 °N, 11.63 °E 57.42 °N, 11.63 °E 57.42 °N, 12.03 °E 57.03 °N, 12.03 °E	Total: 3,680
Averaging for each unique combination of substance, year and month	
CB28	20
CB52	20
CB101	20
CB118	20
CB138	20
CB153	20
CB180	20
DDTPP	19
HCHA	20
HCHG	15
HCB	20
	Total: 214

Table D.4 *Selection criteria for physical-chemical variables in the Kattegat. Search in ICES database, accessed on 1st October 2013.*

In ICES	**Amount of data**
Parameters measured in sediment	
Nitrate	89,145
Nitrite	56,241
Ammonia	50,818
Phosphate	33,551
Temperature	45,255
Chlorophyll a	31,452
	Total: 308,275
Timing between 1990 and 2010 Measured at water surface Consider detection limits Region (1144 km²) between 57.02 °N, 11.63 °E 57.42 °N, 11.63 °E 57.42 °N, 12.03 °E 57.03 °N, 12.03 °E	Total: 20,892
Averaging for each unique combination of substance, year and month	
Nitrate	207
Nitrite	207
Ammonium	207
Phosphate	207
Temperature	207
Chlorophyll a	207
	Total: 1242

Bioaccumulation model (redrafted from De Laender et al., 2011a)

The bioaccumulation model of Hendriks et al. (2001) was used to estimate bioaccumulation factors (BAFs) of PCBs and pesticides. The central calculation in the bioaccumulation model estimates a BAF (L kg^{-1} wet weight) of a species at a given trophic level (BAF_{TL}). The BAF_{TL} is the ratio of the tissue concentration (C_{tissue} µg kg^{-1} wet weight) and the water concentration (C_{water} µg L^{-1}) (Eq. D.1). More specifically, the bioaccumulation model considers pollutant absorption from water $k_{0,x,in}$ (Eq. D.2), pollutant assimilation from ingested food $k_{1,x,in}$, (Eq. D.3), pollutant excretion rate constant to the water $k_{0,x,out}$ (Eq. D.4), pollutant loss via faeces $k_{1,x,out}$ (Eq. D.5), dilution of the pollutant in the body caused by growth or reproduction $k_{2,x,out}$ (Eq. D.6) and the rate constant of biotransformation $k_{3,x,out.}$ Biotransformation rate constants $k_{3,x,out}$ were estimated from De Laender et al. (2011).

$$BAF_{TL} = \frac{C_{tissue}}{C_{water}} = \frac{k_{0,x,in} + (k_{1,x,in} \cdot BAF_{TL-1})}{k_{0,x,out} + k_{1,x,out} + k_{2,x,out} + k_{3,x,out}} \quad \text{(Eq. D.1)}$$

$$k_{0,x,in} = \frac{w^{-\kappa}}{\rho_{H2O} + \frac{\rho_{CH2,TL}}{K_{ow}} + \frac{1}{y_0}} \quad \text{(Eq. D.2)}$$

$$k_{1,x,in} = \frac{p_1}{1 - p_1} \cdot \frac{1}{p_{CH2,TL-1}(K_{ow} - 1) + 1} \cdot \frac{w^{-\kappa}}{\rho_{H2O,1} + \frac{\rho_{CH2,TL}}{q_T \cdot K_{ow}} + \frac{1}{p_{CH2,TL-1} \cdot K_{ow}(1 - p_1) q_T \cdot y_1}} \quad \text{(Eq. D.3)}$$

$$k_{0,x,out} = \frac{1}{p_{CH2,TL-1}(Kow - 1) + 1} \cdot \frac{w^{-\kappa}}{\rho_{H2O,0} + \frac{\rho_{CH2,TL}}{K_{ow}} + \frac{1}{y_0}} \quad \text{(Eq. D.4)}$$

$$k_{1,x,out} = \frac{1}{p_{CH2,TL}(K_{ow} - 1) + 1} \cdot \frac{w^{-\kappa}}{\rho_{H2O,1} + \frac{\rho_{CH2,TL}}{q_T \cdot K_{ow}} + \frac{1}{p_{CH2,TL-1} \cdot K_{ow}(1 - p_1) q_T \cdot y_1}} \quad \text{(Eq. D.5)}$$

$$k_{2,x,out} = q_T \cdot y_2 \cdot w^{-\kappa} \quad \text{(Eq. D.6)}$$

The values of the parameters used in the equations D.2 to D.6 as well as additional species-specific information (weights, lipid content, trophic level) are listed in Table D.5. Substances specific information of the PCBs and pesticides included in the study is provided in Table 1.1.

Table D.5 *Parameters and variables of the bioaccumulation model.*

Parameter	**Scientific name**			**Unit**	**Parameter code**	**Reference**
	Mallotus villosus	*Gadus morhua*	*Gadus morhua*			
Common name	Capelin	Cod (≤ 2 years old)	Cod (> 3 years old)			
Weight (kg)	0.050	1.00	5.00	kg	w	(1)
Trophic level	2	2	3			(2)
Fraction of food assimilated	0.4	0.6	0.8	kg. kg^{-1}	p1	(2)
Body lipid fraction	0.137	0.026	0.026	-	$p_{CH2,TL}$	(3)
Food lipid fraction	0.10	0.10	0.137	-	$p_{CH2,TL-1}$	(3)
Water layer diffuse resistance (absorption)	$2.8 * 10^{-3}$	$2.8 * 10^{-3}$	$2.8 * 10^{-3}$	d .kg^{-1}	$\rho_{H2O,0}$	(4)
Water layer diffuse resistance (assimilation)	$1.1 * 10^{-5}$	$1.1 * 10^{-5}$	$1.1 * 10^{-5}$	d .kg^{-1}	$\rho_{H2O,1}$	(4)
Lipid layer permeation resistance	68	68	68	d .kg^{-1}	ρ_{CH2}	(4)
Food ingestion coefficient	0.005	0.005	0.005	kg^{K} .kg^{-1}	y1	(2)
Biomass production coefficient	0.0006	0.0006	0.0006	kg^{K} .kg^{-1}	y2	(2)
Temperature correction factor	1	1	1	kg .kg^{-1}	q_T	(4)
Water absorption-excretion coefficient	200	200	200	kg^{K} .kg^{-1}	y0	(4)
Rate exponent	0.25	0.25	0.25	-	k	(4)

Table D.6 *Model configurations and model performance of the additive models used to infer time trends for the water temperature, phosphate concentration, nitrate concentration, nitrite concentration, ammonium concentration and polychlorinated biphenyl (PCBs) and pesticide concentrations in the study region located in the Belgian coastal zone (BCZ). Models were assessed by means of the Akaike Information Criterion (AIC) and adjusted correlation coefficient (R^2). P-values indicate whether the covariates (i.e. year, month and chemical) significantly contribute to the model fit (at the 5% level)*

Variable	Year	Month	Chemical	AIC	Deviance explained	R^2 adjusted
PCB & pesticide	$< 2.0 * 10^{-16}$	$1.6 * 10^{-5}$	$< 2.0 * 10^{-16}$	211	70.2	0.66
Temperature	$7.3 * 10^{-1}$	$6.8 * 10^{-11}$	/	-19	61.7	0.58
Phosphate	$1.3 * 10^{-1}$	$3.7 * 10^{-14}$	/	-36	68.9	0.65
Nitrate	$1.2 * 10^{-1}$	$2.1 * 10^{-8}$	/	-29	55.2	0.52
Nitrite	$5.0 * 10^{-3}$	$3.6 * 10^{-4}$	/	-18	47.2	0.41
Ammonium	$4.4 * 10^{-2}$	$3.0 * 10^{-2}$	/	67	30.3	0.24

Table D.7 *Model configurations and model performance of the additive models used to infer time trends for the water temperature, phosphate concentration, nitrate concentration, nitrite concentration, ammonium concentration and polychlorinated biphenyl (PCBs) and pesticide concentrations in the study region located in the Kattegat. Models were assessed by means of the Akaike Information Criterion (AIC) and adjusted correlation coefficient (R^2). P-values indicate whether the covariates (i.e. year, month and chemical) significantly contribute to the model fit (at the 5% level).*

Variable	Year	Month	Chemical	AIC	Deviance explained	R^2 adjusted
PCB & pesticide	$< 2.0 * 10^{-16}$	$2.7 * 10^{-2}$	$< 2.0 * 10^{-16}$	34	84.9	0.83
Temperature	$1.2 * 10^{-3}$	$< 2.0 * 10^{-16}$	/	725	95.2	0.95
Phosphate	$2.1 * 10^{-3}$	$< 2.0 * 10^{-16}$	/	-120	80.9	0.79
Nitrate	$3.7 * 10^{-11}$	$< 2.0 * 10^{-16}$	/	212	77.5	0.76
Nitrite	$2.8 * 10^{-4}$	$< 2.0 * 10^{-16}$	/	161	62.3	0.59
Ammonium	$8.2 * 10^{-11}$	$< 2.0 * 10^{-16}$	/	109	50.1	0.47

Table D.8 *Parameterizations of the nutrient-phytoplankton-zooplankton (NPZ) model, including the abbreviations used per parameter, their maximum and minimum values, units and sources; (5) Billen and Garnier (1997); (6) Lee et al. (2002); (7) Besiktepe et al. (2003); (8) MacLeod et al. (2004); (9) Xu and Hood (2006); (10) Kishi et al. (2007); (11) Soetaert and Herman (2009); (12) Fan and Lv (2009); (13) Cropp and Norbury (2010); (14) Brandt and Wirtz (2010); (15) Ruzicka et al. (2011); (16) Arndt et al. (2011); (17) De Laender et al. (2011).*

Parameter	Abbreviations	Minimum value	Maximum value	Unit	Sources
Critical body burden residu	ECXp	2	8	mmol X kg lipid^{-1}	6,8
Half saturation constant for dissolved inorganic nitrogen limitation	ksDIN	0.25	5	mmolN m^{-3}	5,7,9,10,11,12,13,14,15,16
Half saturation constant for phosphorus limitation	ksP	0.2	0.5	mmolP m^{-3}	5,16
Maximum nitrogen uptake of phytoplankton	maxUptake	0.25	1.5	day^{-1}	10,11,12,13,15
Half saturation constant for photosynthetically active radiation limitation	ksPAR	30	250	µEinst m^{-2} s^{-1}	11
Maximum grazing rate of zooplankton on phytoplankton	maxGrazing	0.8	1	day^{-1}	5,7,10,11,12
Half saturation constant for grazing of zooplankton on phytoplankton limitation	ksGrazing	1	4	mmolN m^{-3}	5,11,14
Fraction of nitrogen lost due to faeces	pFaeces	0.2	0.5	-	11
Intrinsic excretion rate	ExcretationRate	0.1	0.2	day^{-1}	11,12,15
Intrinsic mortality rate	mortailityRate	0.25	0.5	mmolN m^{-3} day^{-1}	5,9,10,11,12,13,15
Amount of nitrogen in chlorophyll a	chlNratio	1	5	mg CHFLa mmolN^{-1}	7,11,15
Slope of the concentration-effect function	slopeP	1	3	-	17
Temperature observed	Tobs	7	12	°C	5,14,16

Table D.9 *Results of pairwise Wilcoxon rank-sum test (α = 0.05) of model deviation distributions for four nutrient-phytoplankton-zooplankton (NPZ) model configurations in the Belgian part of the North Sea: no POPs present ('0TOX'), monitored POP concentrations ('1TOX'), a ten-fold of the monitored POP concentrations ('10TOX') and a hundred-fold of the monitored POP concentrations ('100TOX'). Reported values are model-based p-values of the Wilcoxon rank-sum test pairwise comparing the distributions of two model configuration*

	0TOX	1TOX	10TOX
0TOX	/		
1TOX	$0.9 * 10^{-1}$	/	
10TOX	$1.2 * 10^{-9}$	$2.7 * 10^{-9}$	/
100TOX	$< 2.2 * 10^{-16}$	$< 2.2 * 10^{-16}$	$3.5 * 10^{-5}$

Table D.10 *Results of pairwise Wilcoxon rank-sum test (α = 0.05) of model deviation distributions for four nutrient-phytoplankton-zooplankton (NPZ) model configurations in the Kattegat: no POPs present (0TOX), monitored POP concentrations ('1TOX'), a ten-fold of the monitored POP concentrations ('10TOX') and a hundred-fold of the monitored POP concentrations ('100TOX').Reported values are model-based p-values of the Wilcoxon rank-sum test pairwise comparing the distributions of two model configuration*

	0TOX	1TOX	10TOX
0TOX	/		
1TOX	$< 2.2 * 10^{-16}$	/	
10TOX	$< 2.2 * 10^{-16}$	$< 2.2 * 10^{-16}$	/
100TOX	$< 2.2 * 10^{-16}$	$9.2 * 10^{-4}$	$< 2.2 * 10^{-16}$

References for supportive information

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Summary

A complex chemical cocktail, with unknown composition and concentrations, is present in marine waters. Although the awareness of the vulnerability of marine ecosystems to pollution-induced changes increased, the ecotoxicological effects of chemical pollutants on marine ecosystems are poorly understood. Even in intensively monitored regions such as the North Sea, current knowledge of the ecotoxicological effects of chemicals is limited to few (priority) substances and few (model) species (discussed in **chapter I**). To partly address this knowledge gap, in the present work, using phytoplankton, it is assessed how marine ecosystems respond to the presence of organic chemicals. By analyzing existing data and performing laboratory experiments, ecotoxicological effects of organic chemicals to marine organisms and ecosystem functions are quantified. Specific aims of this work are: (1) to infer spatiotemporal trends of concentrations of organic chemicals; (2) to investigate the impact of primary and secondary emissions on the spatiotemporal trends of organic chemicals; (3) to examine the partitioning of organic chemicals in different environmental compartments; (4) to assess the potential ecotoxicological effect of realistic mixtures of organic chemicals along environmental gradients; and (5) to quantify the relative contribution of organic chemicals to the phytoplankton growth dynamics.

Spatiotemporal trends of polychlorinated biphenyl (PCB) concentrations are inferred based on an extensive set of concentrations monitored between 1991 and 2010 in sediments of the Belgian Coastal Zone (BCZ) and the Western Scheldt estuary in **chapter II**. The time trends unravel two to three-fold PCB concentration decreases in the BCZ during the last 20 years. In the Western Scheldt estuary, time trends are spatially heterogeneous and not significantly decreasing. These results demonstrate that international efforts to cut down emissions of PCBs have been effective to reduce concentrations in open water ecosystems like the BCZ but had little effect in the urbanized and industrialized area of the Scheldt estuary. Most likely, estuaries are subject to secondary emissions from historical pollution.

In **chapter III**, trends found for the BCZ (chapter II) are confirmed at larger spatiotemporal scales. In chapter III multidecadal field observations (1979–2012) in the North Sea and Celtic Sea are analyzed to infer spatiotemporal concentration trends of PCBs in mussels (*Mytilus edulis*) and in sediments. Decreasing interannual PCB concentrations are found in North Sea sediments and mussels. PCB concentrations in sediments show, less than PCB levels in mussels, decreasing interannual trends. In addition in **chapter III**, interannual changes of PCB concentrations are separated from seasonal variability. By doing so, superimposed to the generally decreasing interannual trends, seasonally variable PCB concentrations are observed. These seasonal variations are tightly coupled with seasonally variable chlorophyll a concentrations and organic carbon concentrations. Indeed, the timing of phytoplankton blooms in spring and autumn corresponds to the annual maxima of the organic carbon content and the PCB concentrations in sediments. These results demonstrate the role of seasonal phytoplankton dynamics (biological pump) in the environmental fate of PCBs at large spatiotemporal scales.

The latter is a novel result since the working of the biological pump was never assessed before based on field data collected at the scale of a regional sea in multiple decades.

Despite the generally decreasing spatiotemporal trends of PCBs that are found in chapter II and III, it is not clear whether current concentrations (still) pose a risk to marine ecosystems. In **chapter IV**, the spatiotemporal trends inferred in chapter III are used to assess the ecological risk of PCBs in North Sea and Celtic Sea sediments and mussels. To do so, PCB concentrations are compared with environmental assessment criteria (EAC). It is found that the potential ecotoxicological risk of PCBs change considerably over time and in space. Risk quotients (RQs) of PCBs in marine sediments primarily depend on the location of the monitoring site, i.e. the closer to the coast, the higher the RQ. Especially in summer, when PCB concentrations in sediments are high, PCBs present in marine coastal sediments may pose an environmental risk. By contrast, RQs in mussel depend first on the interannual changes of PCB concentrations. At present, in the Celtic Sea, RQs in mussels are below the value of 1, suggesting no potential environmental risk. In the North Sea, however, PCBS in mussels may still exceed the prescribed environmental quality criteria. Overall, the results shown in chapter IV demonstrate that the spatiotemporal variability in PCB concentrations should be considered in future environmental risk assessments.

Comparing concentrations of chemicals with quality thresholds (as in chapter IV) only suggest a potential ecological risk. Therefore, in case if risk quotients exceed the value of 1, additional assessments are recommended. Considering the results obtained in chapter IV, in **chapter V,** additional experimental studies are performed in which a marine diatom is exposed to a realistic mixture of organic contaminants. To do so, passive samplers are used to achieve exposure to realistic mixtures of organic chemicals close to ambient concentrations. The main conclusion is that organic chemicals present in Belgian marine waters do not affect the intrinsic growth rate of Phaeodactylum tricornutum. In this context, caution is needed when extrapolating these results to field conditions. In the present research, results were obtained under laboratory controlled conditions with one single species and thus neglecting possible species interactions. Therefore, prior to extrapolating these results to other diatoms and other groups of phytoplankton species, it is suggested to assess the validity of the results in a mesocosm experiment (including multiple species and different trophic levels) or under field conditions. In addition, in **chapter V**, the relative contribution of organic chemicals to the growth of a marine diatom is examined. Natural drivers such as nutrients regime, light intensity and temperature explain about 85% of the observed variability in the experimental data.

Although the methodology used in chapter V is a standard way to assess toxicity of chemicals, it is not realistic to use just one algal species to represent ecotoxicological effects of an entire phytoplankton community. Therefore in **chapter VI**, an ecosystem model is used to assess the potential adverse effects of organic contaminants on the total primary production. To do so, we

model phytoplankton dynamics using four classical drivers (light and nutrient availability, temperature and zooplankton grazing) and test whether extending this model with a POP-induced phytoplankton growth limitation term improves model fit. As inclusion of monitored concentrations of PCBs and pesticides did not lead to a better model fit, it is suggested that POP-induced growth limitation of marine phytoplankton in the North Sea and the Kattegat is small compared to the limitations caused by the classical drivers. The inferred contribution of POPs to phytoplankton growth limitation is about 1% in Belgian coastal waters, but in the Kattegat POPs explain about 10% of the phytoplankton growth limitation. These results suggest that there are regional differences in the contribution of POPs to the phytoplankton growth limitation. The validity of these conclusions should be further assessed for other substances, other species and higher trophic levels.

Samenvatting

Een complexe chemische cocktail met ongekende samenstelling en ongekende concentraties van chemische stoffen is aanwezig in het mariene milieu. Ondanks de bewustwording van de kwetsbaarheid van mariene ecosystemen voor chemische verontreiniging zijn de ecotoxicologische effecten van chemische stoffen op mariene ecosystemen weinig bestudeerd. Zelfs in intensief bemonsterde regio's zoals de Noordzee is de huidige kennis over de ecotoxicologische effecten van persistent organische stoffen beperkt tot enkele (prioritaire) stoffen en enkele (model) organismen. Tegen deze achtergrond (gedetailleerd besproken in **hoofdstuk I**) wordt in deze studie, met behulp van fytoplankton, onderzocht hoe mariene ecosystemen reageren op de aanwezigheid van persistente organische stoffen. Door integratie van bestaande veldgegevens en experimentele data werden de ecotoxicologische effecten van organische stoffen op mariene organismen en ecosysteemfuncties gekwantificeerd. Meer specifiek waren de doelen van het onderzoek om: (1) de spatiotemporele trends van de concentraties van organische stoffen af te leiden; (2) de impact van primaire en secondaire emissies op de spatiotemporele trends van organische stoffen te onderzoeken; (3) de partitionering van organische stoffen in diverse milieucompartimenten te bestuderen; (4) de potentiële ecotoxicologische effecten van realistische mengsels van organische stoffen langsheen een milieugradiënt te evalueren; en (5) de relatieve contributie van organische stoffen op de groei van fytoplankton te kwantificeren.

De spatiotemporele trends van polychloorbifenyl (PCB) concentraties werden afgeleid op basis van bestaande veldgegevens gemeten tussen 1991 en 2010 in het sediment van het Belgisch deel van de Noordzee (BCZ) en het Westerschelde estuarium (**hoofdstuk II)**. Deze tijdsreeksen tonen aan dat de PCB concentraties in het BCZ de voorbije twee decennia twee- tot drievoudig gedaald zijn. In de Westerschelde echter zijn de gevonden tijdtrends heterogeen en niet significant dalend. Deze resultaten suggereren dat de internationale inspanningen om PCB emissies te reduceren vooral effect hebben in open zee, maar slechts weinig invloed hebben in het geürbaniseerde en geïndustrialiseerde estuarium van de Westerschelde. Vermoedelijk is het estuarium onderworpen aan secundaire emissies ten gevolge van historische vervuiling.

In **hoofdstuk III** werden de trends gevonden voor het BCZ (zie hoofdstuk II) bevestigd voor een ruimere spatiotemporele schaal. In hoofdstuk III werden veldgegevens (1979-2012) uit de Noordzee en de Keltische Zee geanalyseerd en spatiotemporele trends van de PCB concentraties in mosselen (*Mytilus edulis*) en sediment afgeleid. De PCB concentraties in het sediment toonden, minder dan PCB concentraties in mosselen, dalende inter-jaarlijkse trends. Verder werden in **hoofdstuk III** de inter-jaarlijkse veranderingen van de PCB concentraties gescheiden van de intra-jaarlijkse variabiliteit. Zodoende werd bovenop de, over het algemeen dalende inter-jaarlijkse trends, ook seizoenale verschillen in de PCB concentraties geobserveerd. Deze seizoenale variaties blijken sterk gerelateerd aan de seizoenale schommelingen van chlorofyl a en organische koolstof concentraties. Inderdaad, de momenten van de fytoplanktonbloei in de lente en herfst vallen samen met jaarlijkse maximum concentraties van organische koolstof en

PCB concentraties in het sediment. Deze resultaten tonen het belang aan van de seizoenale fytoplanktondynamiek (biologische pomp) in het partitionering van PCBs (en bij uitbreiding andere persistente organische stoffen) in de verschillende milieucompartimenten. Dit resultaat is vernieuwend omdat de werking van de biologische pomp nooit eerder werd getoond op dergelijke grote ruimtelijke (regionale zee) en temporele (twee tot drie decennia) schalen.

Desondanks de algemeen dalende spatiotemporele trends van PCBs (hoofdstuk II en III), is het onduidelijk of de huidige concentrations een ecologisch risico vormen voor mariene ecosystemen. Daarom werden de spatiotemporele trends, afgeleid in hoofdstuk III, gebruikt in **hoofdstuk IV** om het ecologische risico te evalueren. Daartoe werden de PCB concentraties vergeleken met de voorgeschreven milieukwaliteitsnormen. Het voornaamste resultaat was dat het ecotoxicologische risico van PCBs tijds- en ruimteafhankelijk was. De risicoquotiënten (RQ) van PCBs in marien sediment hangen in de eerste plaats af van de locatie van de monitoring site, i.e. hoe dichter bij de kust hoe hoger de RQ. Bovendien vormen PCBs vooral in de zomermaanden, wanneer de PCB concentraties in het sediment hoog zijn, een mogelijk risico voor het mariene milieu. De potentiële risico's van PCBs in mosselen daarentegen hangen in de eerste plaats af van de inter-jaarlijkse verandering van de PCB concentraties. Alles in beschouwing genomen zijn de RQs in mosselen in de Keltische Zee op dit moment lager dan 1, wat geen potentieel milieurisico suggereert. In de Noordzee daarentegen zijn de PCB concentraties gemeten in mosselen hoger dan de voorschreven milieukwaliteitsnormen. Algemeen gesproken tonen de resultaten uit hoofdstuk VI aan dat de spatiotemporele variabiliteit van de PCB concentraties in zowel sediment als mosselen in beschouwing dienen te worden genomen in toekomstige ecologische risico-evaluaties.

Wanneer concentraties van chemicaliën worden vergeleken met milieukwaliteitsnormen (zoals in hoofdstuk IV) suggereren de resultaten enkel het potentieel ecologische risico, zonder daarbij een causaal verband te leggen. Daarom werden in **hoofdstuk V** bijkomende experimentele studies verricht waarin een mariene diatomee (*Phaeodactylum tricornutum*) werd blootgesteld aan een realistisch mengsel van organische contaminanten. Daartoe werden passieve staalnametechnieken gebruikt om een blootstelling te verkrijgen aan concentraties van organische stoffen dicht bij de realistische, in het milieu voorkomende, concentraties. De resultaten suggereren dat organische contaminanten de groeisnelheid van *P. tricornutum* niet beïnvloeden en dus dat de gemiddelde concentraties aan organische stoffen (althans waarvoor de staalnametechniek affiniteit vertoonden) die gemeten worden in Belgische mariene wateren geen effect hebben op de groei van *P. tricornutum*. In acht genomen dat *P. tricornutum* een intermediair gevoelige soort is binnen de diatomeeën zou men deze conclusie, met enig voorbehoud, kunnen uitbreiden naar andere diatomeeën. Een belangrijke randbemerking is echter dat deze experimentele studie de mogelijke interacties tussen soorten niet in rekening brengt. Bijkomend werd in hoofdstuk V, de relatieve contributie van organische stoffen op de groei van *P. tricornutum* gekwantificeerd. Natuurlijke invloeden zoals het nutriëntenregime, lichtintensiteit

en watertemperatuur verklaren tot 85% van de geobserveerde variabiliteit in de experimentele data.

Ondanks het feit dat de gebruikte methodologie in hoofdstuk V een standaard manier is om het ecotoxicologische effect van chemicaliën te bepalen, is het niet realistisch om slechts één algensoort te gebruiken om de ecotoxicologische effecten van organische polluenten op een volledige fytoplankton gemeenschap te evalueren. Daarom werd in **hoofdstuk VI** een ecosysteemmodel gebruikt om de effecten van organische stoffen op de mariene primaire productie te bepalen. Daartoe werden de dynamiek van de primaire productie gesimuleerd op basis van vier klassieke invloeden (licht- en nutriëntenbeschikbaarheid, watertemperatuur en begrazing door zooplankton). Er werd getest of de uitbreiding van dit model met een pollutiegerelateerde factor de kwaliteit van het model verbeterde. Omdat de integratie van de PCBs en pesticiden in het model niet tot een betere resultaten leidde, was dat de groeilimitatie in de Noordzee en in het Kattegat klein in vergelijking met de limitaties veroorzaakt door de klassieke factoren. Er werd berekend dat de relatieve contributie van persistente organische polluenten op de primaire productie ongeveer 1% was in Belgische mariene wateren en ongeveer 10% van de variabiliteit verklaarde die werd geobserveerd in het Kattegat. Deze resultaten suggereren dus dat er regionale verschillen bestaan in de contributie van organische stoffen op de groei van marien fytoplankton, maar dat het effect van deze stoffen relatief klein is ten opzichte van de natuurlijke factoren (nutriënten, licht, temperatuur en zooplankton). De validiteit van deze resultaten dient verder te worden geëvalueerd voor bijkomende chemische stoffen, andere mariene organismen en hogere trofische niveaus.

Curriculum vitae

Personalia

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2009-present	Ghent University, Doctor in Applied Biological Sciences Faculty Bioscience engineering, Ghent University Thesis: 'Potential risk of organic micropollutants on marine phytoplankton in the greater North Sea: integration of modelling and experimental approaches' Promotors: Prof. Dr. ir. Peter Goethals en Prof. Dr. Colin Janssen
2008-2009	Master Environmental sanitation (Great distinction) Faculty Bioscience engineering, Ghent University Thesis: 'Ecologische impactanalyse van invasieve macro-invertebraten in Vlaamse kreken' Promotors: Prof. Dr. ir. Peter Goethals en Dr. Koen Lock
2005-2008	Master Bio-Engineer (Distinction) Faculty Bioscience engineering, Ghent University Thesis: 'Toegepast etnobotanisch onderzoek: marktonderzoek over wilde eetbare planten te Kisangani (D.R. Kongo, Oostprovincie)' Promotor: Prof. Dr. ir. Patrick Van Damme
2003-2005	Bachelor Bio-Engineer (Distinction) Faculty Bioscience engineering, Ghent University
1997-2003	Secondary education, Science-Mathematics (Distinction) Heilige-Maagd-College, Dendermonde

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English	very good reading, speaking and writing
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Working experience

2009-present	Assisting academic staff, Department of Applied ecology and environmental biology, Faculty Bioscience Engineering, Ghent University

Scientific activities: Publications

- **A1 publications** listed in the Web of Science (newest to oldest)
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- **Gert Everaert**, Pieter Boets, Koen Lock and Peter Goethals. Application of decision trees to analyze the ecological impact of invasive species in polder lakes in Belgium. Presented at the International Society for Ecological Modelling Conference, 6-9 October 2009, Quebec City, Canada.

- Co-author (newest to oldest)
  - Javier Holguin-Gonzalez, **Gert Everaert**, Lorenzo Benedetti, Youri Amerlinck, Peter L.M. Goethals. Use of habitat suitability modeling in the integrated urban water system modeling of the Drava River (Varazdin, Croatia). Presented at the 9th International symposium on Ecohydraulics, 17-21 September 2012, Vienna, Austria.
  - Javier Holguin-Gonzalez, **Gert Everaert**, Lorenzo Benedetti, Peter L.M. Goethals. Integrated ecological modelling for decision support in the integrated urban water system modelling of the Drava river (Varazdin, Croatia). Presented at the 6th Biennial meeting of the International Congress on Environmental Modelling and Software (iEMSs 2012): Managing resources of a limited planet: pathways and visions under uncertainty, 1-5 July 2012, Leipzig, Germany.
  - Javier Holguin-Gonzalez, **Gert Everaert**, Lorenzo Benedetti, Youri Amerlinck, Peter L.M. Goethals. Use of multivariate statistics and machine learning techniques for integrated ecological modelling and decision support in river management. Presented at the Belgian-Dutch conference on machine learning, 24-25 May 2012, Ghent, Belgium.
  - Rahmat Zarkami, Ine S. Pauwels, **Gert Everaert**, Ans Mouton, Peter L.M. Goethals. Comparison of data mining methods for pike (*Esox lucius* L.) habitat suitability modelling in

rivers. Presented at 7th International Congress of the Ecological Informatics Society, 13-16 December 2010, Ghent, Belgium.

- Ine S. Pauwels, **Gert Everaert**, Peter L.M. Goethals. Integrated river assessment by coupling water quality and ecological assessment models. Presented at the 5th Biennial meeting of the International Congress on Environmental Modelling and Software (iEMSs 2010): Modelling for environment's sake, 5-8 July 2010, Ottawa, ON, Canada.

- Poster presentations (newest to oldest)
  - **Gert Everaert**, Frederik De Laender, Michiel Claessens, Jan Baert, Els Monteyne, Patrick Roose, Peter L.M. Goethals, Colin R. Janssen. Contribution of organic contaminants to marine phytoplankton dynamics: an experimental approach. Presented at the SETAC Europe 25th Annual Meeting, 3-7 May 2015, Barcelona, Spain.
  - **Gert Everaert**, Frederik De Laender, Peter L.M. Goethals, Colin R. Janssen. Coupling between phytoplankton and PCB dynamics revealed based on three decades of North Sea and Celtic Sea data. Presented at the SETAC Europe 25th Annual Meeting, 3-7 May 2015, Barcelona, Spain.
  - **Gert Everaert**, Frederik De Laender, Michiel Claessens, Jan Baert, Els Monteyne, Patrick Roose, Peter L.M. Goethals, Colin R. Janssen. Much to do about nothing? Assessing the toxicity of realistic marine contaminant mixtures. Presented at the 15th VLIZ Young Marine Scientists' Day 2015, 20 February 2015, Bruges, Belgium.
  - **Gert Everaert**, Frederik De Laender, Michiel Claessens, Jan Baert, Els Monteyne, Patrick Roose, Peter L.M. Goethals, Colin R. Janssen. Effect of persistent organic pollutants on the marine primary production: an experimental approach. Presented at the SETAC Europe 24th Annual Meeting, 11-15 May 2014, Basel, Switzerland.
  - **Gert Everaert**, Frederik De Laender, Klaas Deneudt, Patrick Roose, Jan Mees, Peter L.M. Goethals, Colin R. Janssen. Additive models to quantify environmental POP exposure in marine sediments. Presented at the SETAC Europe 23rd Annual Meeting, 12-16 May 2013, Glasgow, UK.
  - **Gert Everaert**, Frederik De Laender, Klaas Deneudt, Peter L.M. Goethals, Colin R. Janssen. Should anthropogenic influences be incorporated in marine habitat suitability models? Presented at the 11th VLIZ Young Marine Scientists' Day 2011, 25 February 2011, Bruges, Belgium.
  - **Gert Everaert**, Ine S. Pauwels, Annick Schneiders, Jan Breine, Wim Gabriels, Tom D'heygere, Peter L.M. Goethals. Added value of multi-community cascade models to analyze the relation between water quality conditions and ecological status of rivers. Presented at the Netherlands Annual Ecology Meeting, 9-10 February 2010, Lunteren, Netherland.
  - Ine S. Pauwels, **Gert Everaert**, Peter L.M. Goethals. Integrated river assessment by coupling water quality and ecological assessment models. Presented at the Netherlands Annual Ecology Meeting, 9-10 February 2010, Lunteren, Netherland.
  - Céline Termote, Benoît Dhed'a Djailo, **Gert Everaert**, Sarah Haesaert, Patrick Van Damme. Use and Socio-Economic Value of Wild Edible Plants by the Turumbu in the Tshopo District, DR Congo. Presented at Tropentag, 7-9 October 2008, Hohenheim, Germany.

Scientific activities: Projects

- Ine S. Pauwels, **Gert Everaert**, Peter L.M. Goethals (2010). Ecologische voorspellingsmodellen: Onderzoek met behulp van voorspellingsmodellen voor de ecologische toestand van watersystemen als instrument voor de ex ante evaluatie van maatregelenprogramma's. Studie in opdracht van de Vlaamse Milieumaatschappij. VMM.ADD.002.2009, pp. 196.
- Michelle de la Haye, Edwin C. Verduin, **Gert Everaert**, Peter L.M. Goethals, Ine S. Pauwels, Ciska Blom (2011). Scoren met natuurvriendelijke oevers, onderzoek naar het ecologisch functioneren van oevers langs regionale M-typen wateren. Grontmij, Nederland. Rapportnummer 275711 GM-1032497/MDH, pp.88.

Educational activities: Courses

- Supporting the courses of Prof. Dr. ir. Peter Goethals at the Faculty of Bioscience Engineering (Ghent University) from 2009 to 2015 and this for a total of ca. 125 hours per academic year.
  - Environmental Ecology (partim freshwater ecology)
  - Aquatic Ecology (partim freshwater ecology)
  - Aquatische en Terrestrische Ecologie (partim freshwater ecology)
  - Ecology and Environmental Microbiology (partim ecology)
  - Ecotechnology (part related to static models)
  - Natural systems for wastewater treatment
  - Total quality management
  - Waterkwaliteitsbeheer
  - Water quality management
  - Water quality modelling
  - Biological monitoring of aquatic systems
  - Ecotechniek en natuurbouw

- Supporting the courses of Prof. Dr. Colin Janssen at the Faculty of Bioscience Engineering (Ghent University) from 2009 to 2015 and this for a total of ca. 125 hours per academic year.
  - Ecologische Risico-evaluatie
  - Ecological Risk Assessment
  - Toxicologisch onderzoek van milieuverontreiniging
  - Aquatic Ecology (partim marine ecology)
  - Environmental Ecology (partim marine ecology)
  - Aquatische en terrestrische ecologie (partim marine ecology)
  - Kwaliteitszorg en risicoanalyse

Educational activities: Tutoring master thesis students

- Oscar Julian Velasquez Ballesteros (2014-2015). Thesis: Using additive models to infer spatiotemporal trends of PAHs in the marine environment. Master of Environmental

Sanitation, Faculty of Bioscience Engineering, Ghent University. Promotor: Peter L.M. Goethals and Colin Janssen. Tutor: Gert Everaert.

- Damian Mingo Ndiwago (2014-2015). Thesis: Using additive models to infer spatiotemporal trends of PBDEs in the marine environment. Master of Environmental Sanitation, Faculty of Bioscience Engineering, Ghent University. Promotor: Peter L.M. Goethals and Colin Janssen. Tutor: Gert Everaert.
- Natalija Semjonova (2013-2014). Thesis: Ecological assessment of the Guayas river basin in Ecuador. Master of Environmental Sanitation, Faculty of Bioscience Engineering, Ghent University. Promotor: Peter L.M. Goethals. Tutors: Nguyen Thi Hanh Tien, Gert Everaert, Elina Bennetsen.
- Ruben Theunynck (2012-2013). Thesis: Additieve modellering voor de ontwikkeling en analyse van spatiotemporele tijdreeksen van persistente organische polluenten in biota. Master Bio-Engineer, Faculty of Bioscience Engineering, Ghent University. Promotor: Peter L.M. Goethals and Colin Janssen. Tutor: Gert Everaert.
- Ninh Le Thi Hai (2012-2013.)Thesis: Intercontinental comparison of effects of pollution on macroinvertebrate taxa in freshwater. Master of Environmental Sanitation, Faculty of Bioscience Engineering, Ghent University. Promotor: Peter L.M. Goethals and Olivier Thas. Tutor: Gert Everaert
- Rémi Petit (2011-2012). Thesis: Key drivers of primary production in the Baltic Sea: oceanographic variables, chemical pollutants or demographic stress? Master of Environmental Sanitation, Faculty of Bioscience Engineering, Ghent University. Promotor: Colin Janssen and Frederik De Laender. Tutor: Gert Everaert.

Scientific awards

- Best paper award for the best student paper and presentation at the Fifth Biennial meeting of the International Congress on Environmental Modelling and Software (iEMSs 2010): Modelling for environment's sake, 5-8 July 2010, Ottawa, ON, Canada for the paper and presentation of ‘Development of data-driven models for the assessment of macroinvertebrates in rivers in Flanders’. Gert Everaert, Ine S. Pauwels, Peter L.M. Goethals.